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SMALL-MOLECULE INHIBITORS OF INTERLEUKIN-2

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates to small-molecule inhibitors of interleukin-2.

DESCRIPTION OF RELATED ART

The cytokine interleukin-2 is a principal regulator of the Th1, or cell-mediated, immune response [Waldmann et al., "Contrasting Roles of IL-2 and IL-15 in the Life and Death of Lymphocytes: Implications for Immunotherapy", *Immunity*, **2001**, 14, 105-110]. When the body launches a Th1 response against its own cells, autoimmune diseases (such as rheumatoid arthritis, multiple sclerosis, uveitis, and psoriasis) occur. Similarly, cell-mediated immunity causes rejection of transplanted organs (allograft rejection) and graft-versus-host disease (GVHD), a serious complication that can occur after bone-marrow transplantation.

The IL-2 receptor system (IL-2R) contains three subunits. The dimeric receptor, containing the beta and gamma subunits, is found on most immune cells; IL-2-mediated signaling through this receptor stimulates basal cell growth of T-cells, natural killer cells, and macrophages. During a Th1 immune response, the alpha chain of the IL-2 receptor (IL-2Rα) is expressed on the surface of activated T-cells. Binding of IL-2 to this trimeric receptor causes the activated T-cells to proliferate, and this T-cell proliferation is in turn responsible for stimulating the cell-mediated immune response.

Currently used immunosuppressive protocols designed to inhibit allograft rejection and GVHD involve the use of general immunosuppressants such as azathioprine, cyclosporin, rapamycin, tacrolimus, mycophenolate mofetil, and corticosteroids, generally in combinations of two or more of these drugs. All of these can cause toxic side effects to non-lymphoid tissues. It would be desirable to develop inhibitors of the Th1 immune response that selectively block the proliferative activity of IL-2/IL-2Rα binding without affecting the role of IL-2 role in basal cell growth, as these selective IL-2Rα antagonists should be safer than general immunosuppressants.

Recently, two antibodies directed against IL-2Rα (basiliximab and daclizumab) have been approved for allograft rejection. Studies have shown that these antibodies provide benefits over the standard three-drug (azathioprione, cyclosporin and mycophenolate mofetil or steroids) regime without some of the side effects of that therapy (Berard et al., "A review of interleukin-2 receptor antagonists in solid organ transplantation", *Pharmacotherapy* **1999**, *19*, 1127-1137; Nashan, "The interleukin-2 inhibitors and their role in low-toxicity regimens", *Transplantation Proc.* **1999**, *31 (Suppl. 8A)*, 23S-26S). However, these antibodies are not orally bioavailable.

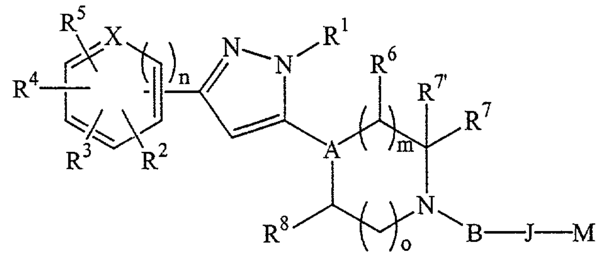
Application of new immunosuppressants to autoimmune disease has lagged behind treatments for graft rejection. While autoimmune diseases have diverse manifestations, the up-regulation of the immune response appears to be a common underlying pathology. Important unmet medical needs exist for autoimmune diseases such as rheumatoid arthritis, multiple sclerosis (MS), uveitis, and psoriasis. Several of the therapeutics currently used to treat autoimmune diseases require intravenous, intramuscular or subcutaneous injection, and are thus suboptimal for chronic use. Nevertheless, several ongoing clinical trials are investigating the use of anti-IL-2Rα antibodies for multiple sclerosis and other autoimmune diseases. See, for example, Brok et al., "Prophylactic and therapeutic effects of a humanized monoclonal antibody against the IL-2 receptor (daclizumab) on collagen-induced arthritis (CIA) in rhesus monkeys", *Clin. Exp. Immunol.* **2001**, *124*, 134-141.

Thus, despite improved therapies for immunosuppression, autoimmune diseases continue to be important pathologies in need of safe and efficacious treatments. To date, no small-molecule IL-2 antagonists have been reported. It would be desirable to develop a small-molecule orally available inhibitor of the IL-2/IL-2Rα interaction.

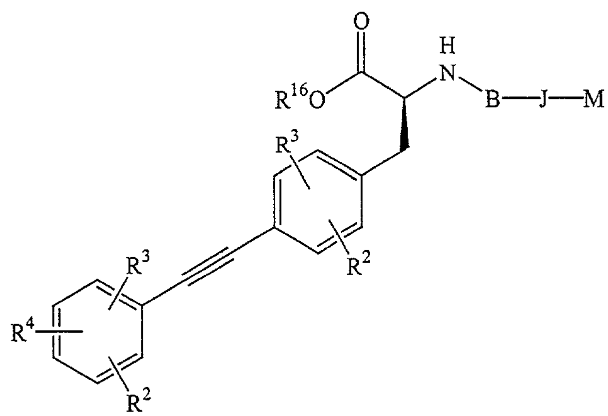
The disclosures of all documents referred to throughout this application are incorporated herein by reference.

SUMMARY OF THE INVENTION

In a first aspect, this invention is compounds of formula I or formula I′



I



I′

where:

m is an integer selected from 0, 1, and 2;

n and o are integers independently selected from 0 and 1;

A is selected from the group consisting of N and CH;

B is selected from the group consisting of $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-NH-$, $-CH_2-O-CH_2-$, $-CH_2-S-CH_2-$, $-C(=O)-NH-$, $-C(=O)-CH_2-$, $-CH_2-C(=O)-NH-$, $-C(=O)-CH_2-C(=O)-$, $-C(=O)-NH-CH_2-$, $-C(=O)-NH-$, $-S(=O)-$, $-S(=O)_2-$,$-S(=O)-NH-$, $-S(=O)_2-NH-$, $-S(=O)-CH_2-$, $-S(=O)_2-CH_2-$, $-S(=O)-CH_2-NH-$, $-S(=O)_2-CH_2-NH-$, $-S(=O)_2-NH-CH_2-$, $-CH_2-S(=O)_2-NH-$, $-C(=O)-NH-S(=O)_2-$, $-S(=O)_2-NH-C(=O)-$, $-C(=O)-CH_2-S(=O)_2-$, and $-S(=O)_2-CH_2-C(=O)-$;

J is absent or is selected from the group consisting of $-O-$, $-S-$, $-CHR^{15}-O-$, $-CH_2-CHR^{15}-O-$, $-NH-$, $-NH-CHR^{15}-$, $-NH-CHR^{15}-C(=O)-$, $-C(=O)-$, $-CH_2-$, $-CHR^{15}-CH_2-NH-$, $-C(=O)-CHR^{15}-$, and $-C(=O)-CHR^{15}-NH-$;

L is selected from the group consisting of $-CH_2-O-$, $-O-CH_2-$, $-CH_2-CH_2-O-$, $-O-CH_2-CH_2-$, $-CH_2-O-CH_2-$, $-CH_2-S-CH_2-$, $-C(=O)-NH-$, $-O-C(=O)-NH-$, $-CH_2-C(=O)-NH-$, $-C(=O)-CH_2-NH-$,

-C(=O)-NH-CH_2-, -NH-C(=O)-, -NH-C(=O)-O-, -NH-CH_2-C(=O)-, -NH-C(=O)-CH_2-, -CH_2-NH-C(=O)-, -NH-C(=O)-NH-, -NH-S(=O)$_2$-NH-, -NH-S(=O)$_2$-, -NH-S(=O)$_2$-CH_2-, -CH_2-NH-S(=O)$_2$-, -S(=O)$_2$-NH-, -S(=O)$_2$-NH-CH_2-, -CH_2-S(=O)$_2$-NH-, -C(=O)-NH-S(=O)$_2$-, -S(=O)$_2$-NH-C(=O)-, -CH_2-NH-, -CH_2-CH_2-NH-, -NH-CH_2-, -NH-CH_2-CH_2-, -CH_2-NH-CH_2-, -C≡C-, -CH_2-C≡C-, -CH_2-CH_2-, -CH_2-CH_2-CH_2-, -CH_2-CH=CH-, -CH=CH-CH_2-, and -CH=CH-;

M is selected from the group consisting of R^9 and an optionally substituted group selected from phenyl, naphthyl, C_3-C_7-cycloalkyl, and heterocyclyl, the heterocyclyl group being aliphatic, partially unsaturated, or aromatic, and containing 1 or 2 rings each containing 5-7 ring atoms of which 0-3 are hetero atoms selected from N, O and S, provided that at least one ring contains a heteroatom and where any ring carbon or sulfur may optionally be oxidized, the optional substituents being up to three groups selected from R^1, R^2, and R^9;

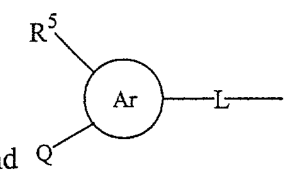
Q is selected from the group consisting of -C(=O)OR^{16}, -C(=O)-NH-C(=O)-CF_3, -C(=O)-NH-S(=O)$_2$-R^2, -C(=O)-NR^1-OH, 5-oxo-4,5-dihydro[1,2,4]oxadiazol-3-yl, and 1*H*-tetrazol-5-yl;

X is A when n is 1 and is CH, N, O or S when n is 0;

R^1 is selected from the group consisting of hydrogen, (C_1-C_6)alkyl, halo-(C_1-C_6)alkyl, and (C_3-C_6)cycloalkyl;

R^2, R^3 and R^5 are individually selected from the group consisting of hydrogen, cyano, nitro, phenyl, phenoxy, benzyl, C_1-C_6alkyl, halo, halo-C_1-C_6alkyl, C_3-C_6cycloalkyl, C_1-C_6alkoxy, hydroxy, C_1-C_2alkoxy-methoxy, hydroxy-C_1-C_6alkyl, formyl, C_1-C_6alkylcarbonyl, amino, C_1-C_6alkylamino, aminocarbonyl, C_1-C_6alkylaminocarbonyl, formylamino, and C_1-C_6alkylcarbonylamino, where any alkyl or phenyl may optionally be substituted with halo or Q;

R^4 is selected from the group consisting of R^2 and



,

where Ar is a homo- or hetero-aryl group having 1 or 2 rings, each ring containing 5, 6 or 7 ring atoms of which 1-3 may be heteroatoms selected from N, O and S;

R^6 is selected from the group consisting of hydrogen, C_1-C_6alkyl, halo, halo-C_1-C_6alkyl, C_3-C_6cycloalkyl, C_1-C_6alkoxy, C_1-C_6alkoxy-C_1-C_6alkyl, hydroxy, hydroxy-C_1-C_6alkyl,

$HC(=O)$-C_1-C_6alkyl, carboxy, carboxy-C_1-C_6alkyl, carbonylamino-C_1-C_6alkyl, aminocarbonyl, (C_1-C_6alkyl)aminocarbonyl, di(C_1-C_6alkyl)aminocarbonyl, and aminocarbonyl-C_1-C_6alkyl; or

R^7 is selected from the group consisting of hydrogen, C_1-C_6alkyl, halo, halo-C_1-C_6alkyl, C_3-C_6cycloalkyl, C_1-C_6alkoxy, C_1-C_6alkoxy-C_1-C_6alkyl, hydroxy, hydroxy-C_1-C_6alkyl, $HC(=O)$-C_1-C_6alkyl, carboxy, carboxy-C_1-C_6alkyl, carbonylamino-C_1-C_6alkyl, aminocarbonyl, (C_1-C_6alkyl)aminocarbonyl, di(C_1-C_6alkyl)aminocarbonyl, and aminocarbonyl-C_1-C_6alkyl;

$R^{7'}$ is hydrogen; or

R^7 and $R^{7'}$ together with the carbon to which they are bonded form $C(=O)$;

R^8 is selected from the group consisting of hydrogen, hydroxy, C_1-C_6alkoxy, C_1-C_6alkyl, halo, halo-C_1-C_6alkyl, and C_3-C_6 cycloalkyl;

R^9 is selected from the group consisting of $-NR^{10}R^{11}$, $-C(=NR^{12})-NR^{13}$, $-N=CR^{14}-NR^{10}R^{11}$, $-NR^{13}-CR^{14}=NR^{12}$, and $-NR^{13}-C(=NR^{12})-NR^{13}$;

R^{10}, R^{11}, R^{12}, R^{13}, and R^{14} are independently selected from the group consisting of hydrogen, hydroxy, hydroxy-C_1-C_6alkyl, C_1-C_6alkyl, halo-C_1-C_6alkyl, C_1-C_6alkoxy, C_1-C_6alkoxy-C_1-C_6alkyl, and C_3-C_7 cycloalkyl; or any member of the group R^{10}, R^{11}, R^{12}, R^{13}, and R^{14} together with the nitrogen to which it is attached forms a 5, 6 or 7 member heterocycle with any other member of the group, the heterocycle optionally containing one additional heteroatom selected from N, O or S;

R^{15} is selected from the group consisting of hydrogen, C_1-C_{12}alkyl, C_3-C_7 cycloalkyl, aminocarbonyl, C_1-C_6alkylaminocarbonyl, and di(C_1-C_6alkyl)aminocarbonyl; and

R^{16} is selected from the group consisting of hydrogen, C_1-C_6alkyl, C_3-C_{13} cycloalkyl, C_6-C_{10}aryl, acetylamino-C_1-C_{12}alkyl, C_1-C_6alkylcarbonyloxy-C_1-C_6alkyl, and C_6-C_{10}aryl-C_0-C_6alkylcarbonyloxy-C_1-C_6alkyl,

and the pharmaceutically acceptable salts thereof;

provided that the compound is not N-[2-[1-(aminoiminomethyl)-3-piperidinyl]-1-oxoethyl]-4-phenylethynyl-phenylalanine methyl ester or a pharmaceutically acceptable salt thereof.

In a second aspect, this invention is pharmaceutical compositions comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of at least one compound of the first aspect of this invention. These compositions find use for the treatment of autoimmune diseases (e.g. rheumatoid arthritis, multiple sclerosis, uveitis, and psoriasis), allograft rejection, and GVHD.

In a third aspect, this invention is a method of treating an animal having disease for which antagonism of IL-2/IL-2R binding is indicated (an interleukin-2 mediated disease), such as autoimmune diseases, allograft rejection, and GVHD, comprising administration to that animal of a therapeutically effective amount of at least one compound of the first aspect of this invention, optionally in conjunction with at least one other conventional therapeutic agent for the disease being treated.

In a fourth aspect, this invention is the use of compounds of the first aspect of this invention in the preparation of medicaments for the treatment of diseases capable of treatment by an antagonist of IL-2/IL-2R binding, such as autoimmune diseases, allograft rejection, and GVHD.

In a fifth aspect, this invention is methods of preparing the compounds of the first aspect of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

"Alkyl" means a linear hydrocarbyl group having the range of carbon atoms specified, or a branched or cyclic hydrocarbyl group having at least 3 carbon atoms within the range of carbon atoms specified. Exemplary alkyl groups include methyl, ethyl, isopropyl, cyclopropyl, *tert*-butyl, cyclopropylmethyl, and hexyl.

"Animal" includes humans and non-human mammals, such as companion animals (cats, dogs, and the like) and farm animals (cattle, horses, sheep, goats, swine, and the like).

"Disease" includes any unhealthy condition of an animal, including injury, particularly interleukin-2 mediated diseases, such as autoimmune diseases (e.g. rheumatoid arthritis, multiple sclerosis, uveitis, and psoriasis), allograft rejection, and graft-versus-host disease.

"Halo" means fluoro, chloro, or bromo.

"Haloalkyl" means alkyl substituted with from 1 to 3 halogen atoms selected from fluorine, chlorine, or bromine.

"Pharmaceutically acceptable excipient " means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

"Pharmaceutically acceptable salts" means salts that are pharmaceutically acceptable and have the desired pharmacological properties. Such salts include salts that may be formed where acidic protons present in the compounds are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, e.g. sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic bases such as the amine bases, e.g. ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (e.g. hydrochloric and hydrobromic acids) and organic acids (e.g. acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). When there are two acidic groups present, a pharmaceutically acceptable salt may be a mono-acid-mono-salt or a di-salt; and similarly where there are more than two acidic groups present, some or all of such groups can be salified.

A "protecting group" has the meaning conventionally associated with it in organic synthesis, i.e. a group that selectively blocks one or more reactive sites in a multifunctional compound such that a chemical reaction can be carried out selectively on another unprotected reactive site and such that the group can readily be removed after the selective reaction is complete.

A "therapeutically effective amount" means the amount that, when administered to an animal for treating a disease, is sufficient to effect treatment for that disease.

"Treating" or "treatment" of a disease includes preventing the disease from occurring in an animal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment), inhibiting the disease (slowing or arresting its development), providing relief from the symptoms or side-effects of the disease (including palliative treatment), and relieving the disease (causing regression of the disease).

The compounds of formula I′ possess a chiral center in the alanine portion of the molecule, where they have the normal (S) configuration. The compounds of this invention may also possess one or

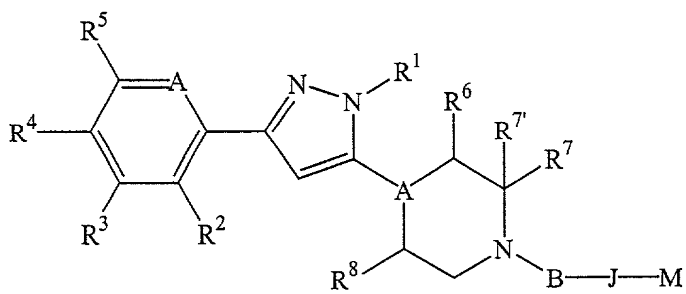
more (additional) chiral centers. Methods for the determination of stereochemistry and the separation of stereoisomers are well known to a person of ordinary skill in the art [*see* the discussion in Chapter 4 of March, "Advanced Organic Chemistry", 4th ed., 1992, John Wiley and Sons, New York, NY].

Implicit hydrogen atoms on carbon and sometimes on nitrogen atoms are generally omitted from the formulae for clarity, but should be understood to be present.

Presently Preferred Compounds

While the broadest definition of the invention is set out in the Summary of the Invention, certain compounds of this invention are presently preferred.

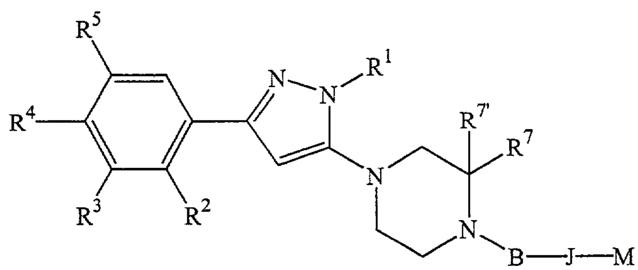
Presently preferred compounds of this invention are compounds of formula I that are compounds of formula II



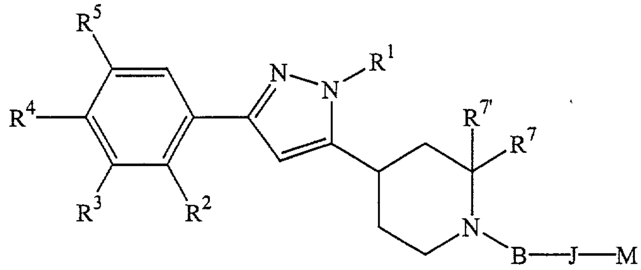
II

where the substituents are as defined for formula I;
and the pharmaceutically acceptable salts thereof.

More preferred compounds are compounds of formula II that are compounds of formula III or formula III′

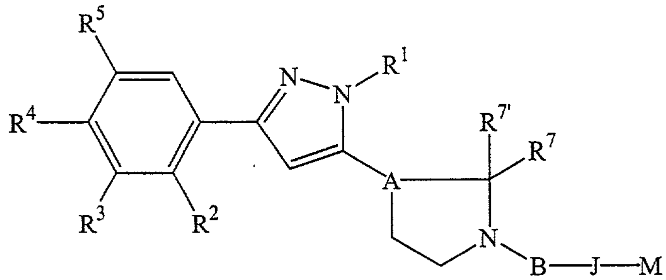


III

 III′

where the substituents are as defined for formula I;
and the pharmaceutically acceptable salts thereof.

Other preferred compounds are compounds of formula I that are compounds of formula IV

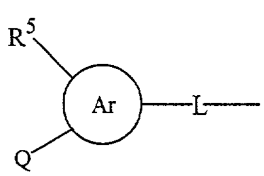
 IV

where the substituents are as defined for formula I;
and the pharmaceutically acceptable salts thereof.

Presently preferred classes of compounds of this invention include those where:

(1) R^1 is hydrogen or lower alkyl, especially methyl;

(2) R^2 and R^3 are hydrogen, lower alkyl, cyano, or halo (especially chloro); and more preferably one or both are chloro;

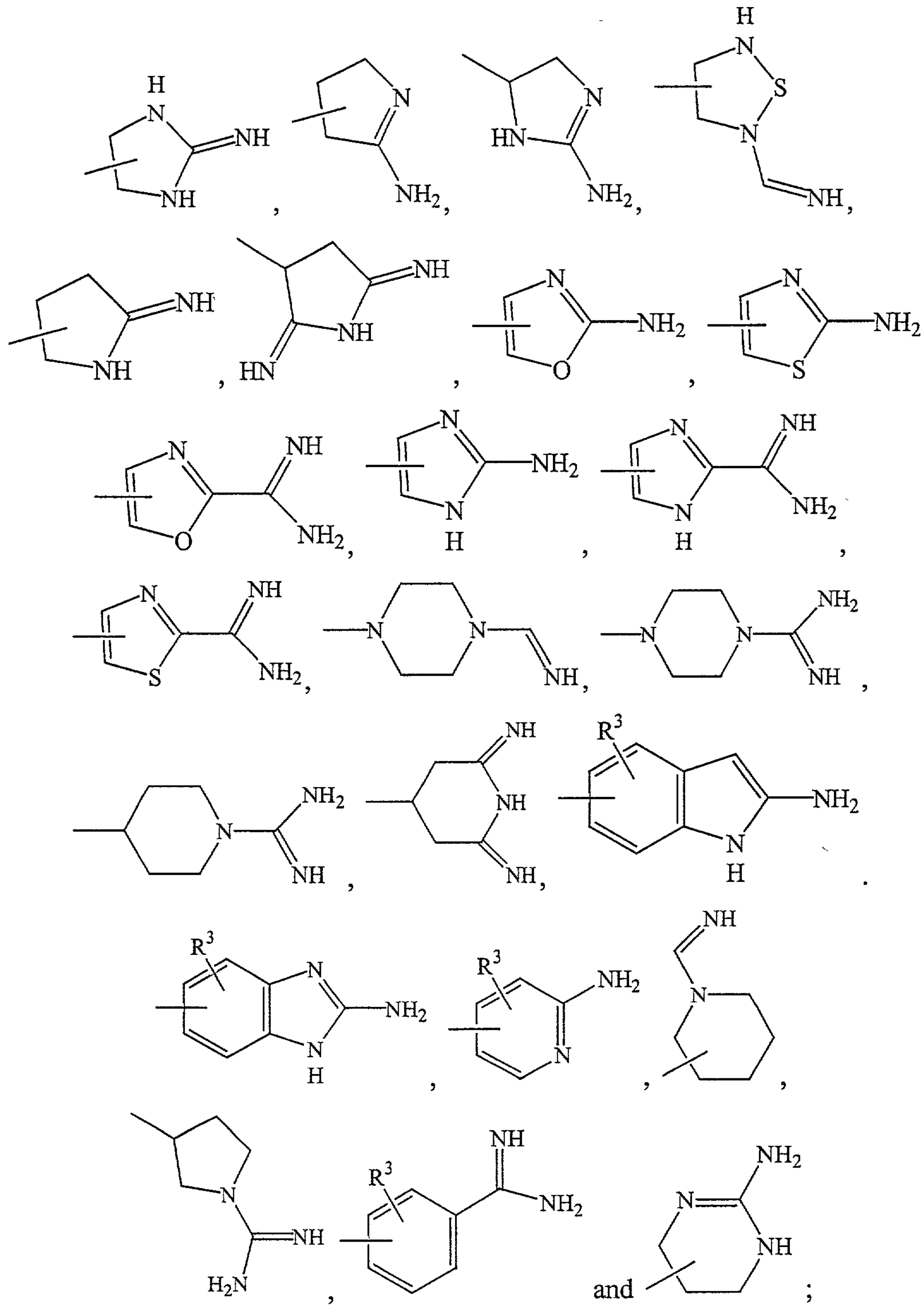
(3) R^4 is , especially where one or more of the following preferences applies: Ar is selected from the group consisting of phenyl, furyl, thienyl, oxazolyl, thiazolyl, and pyrrolyl; R^5 is hydroxy, C_1-C_2alkoxy-methoxy and C_1-C_3-alkoxy; Q is a negatively charged species such as carboxy (or a prodrug thereof) or tetrazole; and L is -O-, -CH_2-O-, -O-CH_2- or -CH_2-CH_2-O-.

(4) B is -C(=O)- or –$S(=O)_2$-.

(5) J is -CH_2-, -CH_2-CH_2-, -NH-, -NH-CH_2-, -CH_2-NH-, -CH_2-NH-C(=O)-, -CH_2-NH-C(=O)-C_1-C_6alkyl- and -CH_2-NH-C(=O)-CH(C_3-C_{12}cycloalkyl)-.

(6) B-J combinations are -C(=O)-CH_2-NH-C(=O)-CH(C_1-C_6alkyl)-, -C(=O)-CH_2-NH-C(=O)-CH(C_3-C_{12} cycloalkyl)-, -C(=O)-NH-(C_2-C_6alkyl)-, -S(=O)$_2$-NH-(C_2-C_6alkyl)-, -C(=O)-NH-, -S(=O)$_2$-NH-, -C(=O)-CH_2- and -S(=O)$_2$-CH_2-.

(7) M is selected from the group consisting of R^9,



;

especially where M is not R^9.

A number of different preferences have been given above, and following any one of these preferences results in a compound of this invention that is more presently preferred than a compound in which that particular preference is not followed (e.g. compounds of formula III or formula III′ are more preferred than compounds of formula II that are not compounds of formula III or formula III′; and compounds of formula II are more preferred than compounds of formula I that are not compounds of formula II). However, substituent preferences in particular are generally independent [although the B-J preferences are not entirely independent of the B preferences and J preferences], and additive; and following more than one of these preferences may result in a more presently preferred compound than one in which fewer of the preferences are followed.

Presently particularly preferred compounds of this invention are the compounds of Examples 1 to 76, and the pharmaceutically acceptable salts thereof.

Pharmacology and Utility

The compounds of this invention are antagonists of IL-2/IL-2R binding. Their activity as antagonists of IL-2/IL-2R binding *in vitro* can be measured by methods such as the scintillation proximity assay described in Example 77 to demonstrate that the compounds inhibit binding of IL-2 to IL-2Rα in a dose-dependent fashion. Other methods, such as ELISA, enzyme-linked protein binding assays, and energy transfer assays, can also be used.

In addition to these assays demonstrating that compounds antagonize binding of IL-2 to IL-2Rα, other measurements can be used to demonstrate directly the binding of compounds to IL-2. Such methods include NMR, x-ray crystallography, analytical ultracentrifugation, surface plasmon resonance (SPR, Biacore), and isothermal calorimetry. By these methods, compounds of this invention can be shown to bind to IL-2 with one-to-one stoichiometry; furthermore, compounds can be shown to bind at the IL-2 surface that is used to bind IL-2Rα.

Cell-based assays to study inhibition of IL-2/IL-2Rα binding use phosphorylation of STAT5 as a marker of IL-2 activity on CTLL-2 cells. When IL-2 binds to its receptor, an intracellular signal is transduced from Jak1 and Jak3 proteins to STAT5. Phosphorylated STAT5 then translocates to the

nucleus and activates transcription. Inhibition of STAT5 phosphorylation therefore indicates that the compounds interfere with IL-2 mediated signal transduction. For selectivity studies, one can monitor STAT5 phosphorylation in response to IL-15 binding. IL-15 is homologous to IL-2 and signals through the IL-2 dimeric receptor. IL-15 does not bind to IL-2Rα, and selective IL-2 inhibitors should not inhibit IL-15 signaling. A description of the assay is given in Example 78.

The activity of compounds of this invention can be measured *in vivo* by activity against animal models of the selected disease.

The therapeutic ratio of a compound can be determined for a selected disease, for example, by comparing the dose that gives effective activity in a suitable *in vivo* model in a suitable animal species, with the dose that gives significant weight loss (or other observable side-effects) in the test animal species.

Pharmaceutical compositions and administration

In general, compounds of this invention will be administered in therapeutically effective amounts by any of the usual modes known in the art, either singly or in combination with at least one other compound of this invention and/or at least one other conventional therapeutic agent for the disease being treated. A therapeutically effective amount may vary widely depending on the disease, its severity, the age and relative health of the animal being treated, the potency of the compound(s), and other factors. A representative dose will range from 0.001 to 100 milligrams per kilogram body weight of the animal per day(mg/Kg/day); for example, from 0.01 to 10 mg/Kg/day. A person of ordinary skill in the art will be able without undue experimentation, having regard to that skill and this disclosure, to determine a therapeutically effective amount of a compound of this invention for a given disease.

In general, compounds of this invention will be administered as pharmaceutical compositions by one of the following routes: oral, topical, systemic (e.g. transdermal, intranasal, or by suppository), or parenteral (e.g. intramuscular, subcutaneous, or intravenous injection). Compositions may take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, suspensions, elixirs, aerosols, or any other appropriate compositions; and comprise at least one compound of this invention in combination with at least one pharmaceutically acceptable excipient. Suitable excipients are well known to persons of ordinary skill in the art, and they, and the methods of formulating the

compositions, may be found in such standard references as Gennaro, ed., "Remington: The Science and Practice of Pharmacy", 20th ed., 2000, Lippincott, Williams & Wilkins, Philadelphia, PA. Suitable liquid carriers, especially for injectable solutions, include water, aqueous saline solution, aqueous dextrose solution, and glycols.

The amount of a compound of this invention in the composition may vary widely depending on the type of composition, size of a unit dosage, kind of excipients, and other factors well known to those of ordinary skill in the art. In general, the final composition may comprise from 0.001 percent by weight (%w) to 90 %w of the compound of this invention, preferably 0.01 %w to 10 %w, with the remainder being the excipient or excipients.

A pharmaceutical composition of this invention may optionally contain, in addition to a compound of this invention, at least one other compound of this invention, and/or at least one pharmaceutically active compound selected from compounds conventionally used in the treatment of the selected disease. For example, in the case of allograft rejection, the composition may contain one or more of the conventional immunosuppressants mentioned in the BACKGROUND TO THE INVENTION.

Additionally, compounds conventionally used in the treatment of the selected disease may also be co-administered with compound(s) of this invention. "Co-administered" here includes administration during the course of treatment with the compound(s) of this invention, and is not limited to administration at the same time as the administration of the compound(s) of this invention, depending on appropriate dosing schedules for the conventional compounds and for the compound(s) of this invention.

Preparation of the Compounds of this Invention

The starting materials and reagents used in preparing these compounds are either available from commercial suppliers such as Aldrich Chemical Company (Milwaukee, WI), Bachem (Torrance, CA), Sigma (St. Louis, MO), or are prepared by methods well known to a person of ordinary skill in the art following procedures described in such references as Fieser and Fieser, "Reagents for Organic Synthesis", vols 1-17, 1991, John Wiley and Sons, New York, NY; Rodd, "Chemistry of Carbon Compounds", vols. 1-5 and supps, 1989, Elsevier Science Publishers; "Organic Reactions", vols 1-40, 1991, John Wiley and Sons, New York, NY; March, "Advanced Organic Chemistry", 4th ed., 1992,

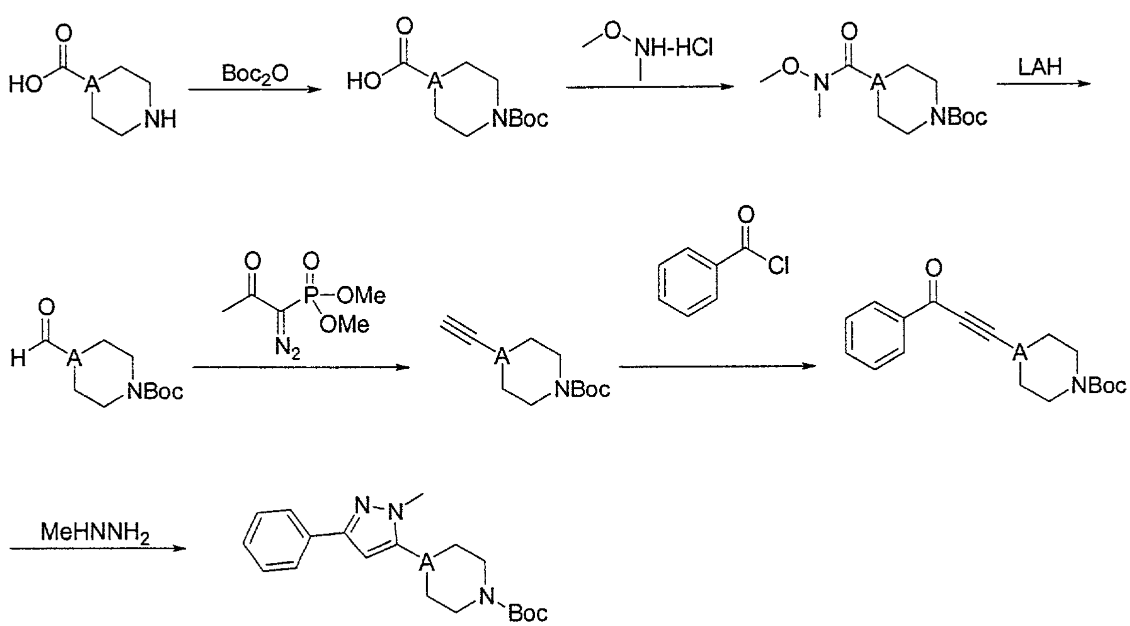
John Wiley and Sons, New York, NY; and Larock, “Comprehensive Organic Transformations”, 1989, VCH Publishers. These schemes are merely illustrative of some methods by which the compounds of this invention can be synthesized, and various modifications to these schemes can be made and will be suggested to a person of ordinary skill in the art having regard to this disclosure.

The starting materials, intermediates, and compounds of this invention may be isolated and purified using conventional techniques, including filtration, distillation, crystallization, chromatography, and the like. They may be characterized using conventional methods, including physical constants and spectral data.

Unless specified to the contrary, the reactions described herein take place at atmospheric pressure over a temperature range between about 0 °C and 125 °C.

The preparation of the core of the compounds of formula I may be accomplished by methods similar to those seen in Examples 16 and other like examples; i.e. as in Scheme I below, where substituents and variations in ring size of the compound of formula I have been omitted for clarity.

Scheme I

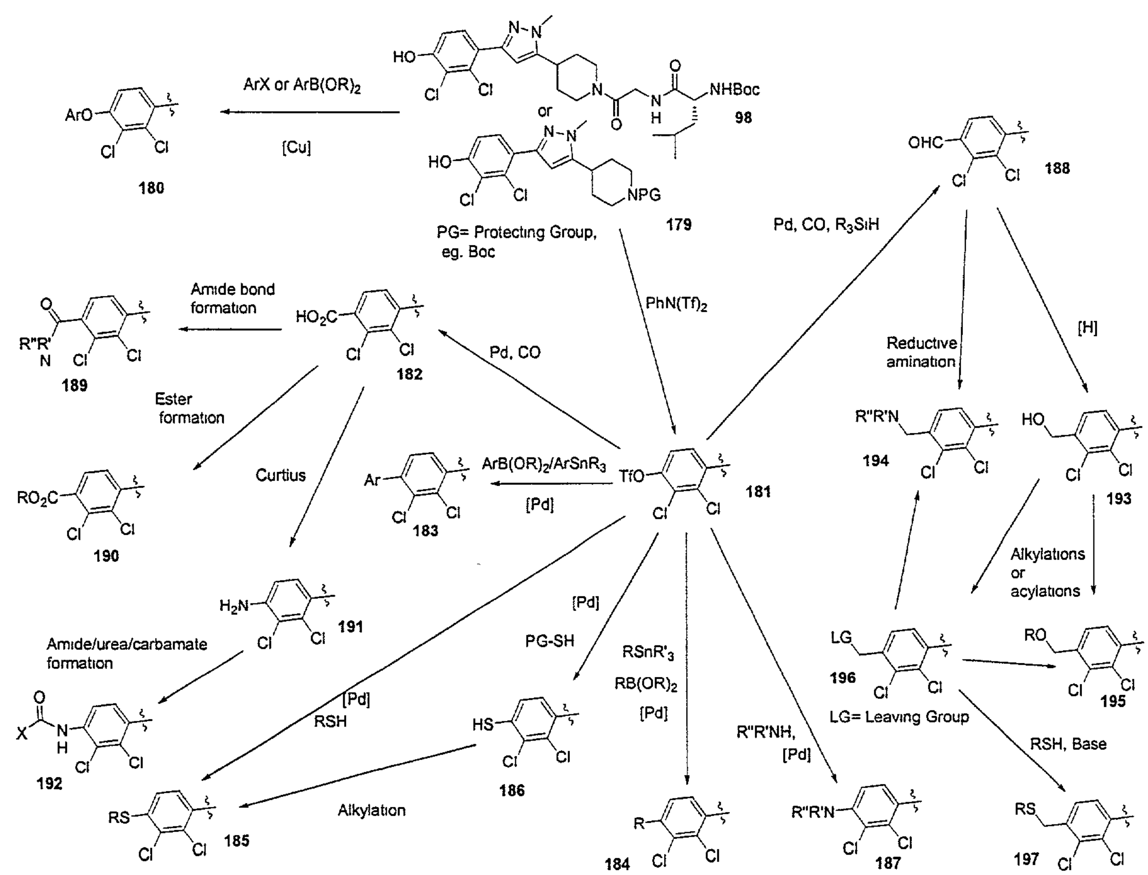


As seen in Scheme I, above, a piperidine or piperazine carboxylic acid is protected at the nitrogen, then reacted with dimethylhydroxylamine, followed by reduction with an agent such as lithium

aluminum hydride to form the corresponding aldehyde, then treated with a reagent such as dimethyl 1-diazo-2-oxopropylphosphonate to convert the aldehyde into an acetylene. Reaction of the acetylene with an aryl chloride gives an arylacetylene, and this is reacted with an alkylhydrazine to form the imidazole center ring. The resulting compound can be deprotected in the conventional manner.

Elaboration of the aryl group can be accomplished by a variety of methods shown in Scheme II:

Scheme II



The phenols **98** and **179** can be used to prepare biaryl ether type analogues **180** using, for example, Ullman type couplings as described by, for example, Evans et al., *Tetrahedron Lett.* **1998**, *39*, 2937-2940, *J. Am. Chem. Soc.* **1997**, *119*, 3395-3396, *J. Am. Chem. Soc.* **1997**, *119*, 10539-10540, and *Tetrahedron Lett.* **1998**, *39*, 2933-2936). Compounds **98** and **179** may also be used to make the corresponding trifluoromethanesulfonyloxy intermediate **181**, using, for example, N-phenyltrifluoro-methansulfonimide in the presence of a base such as triethylamine. The triflated phenol **181** is a valuable

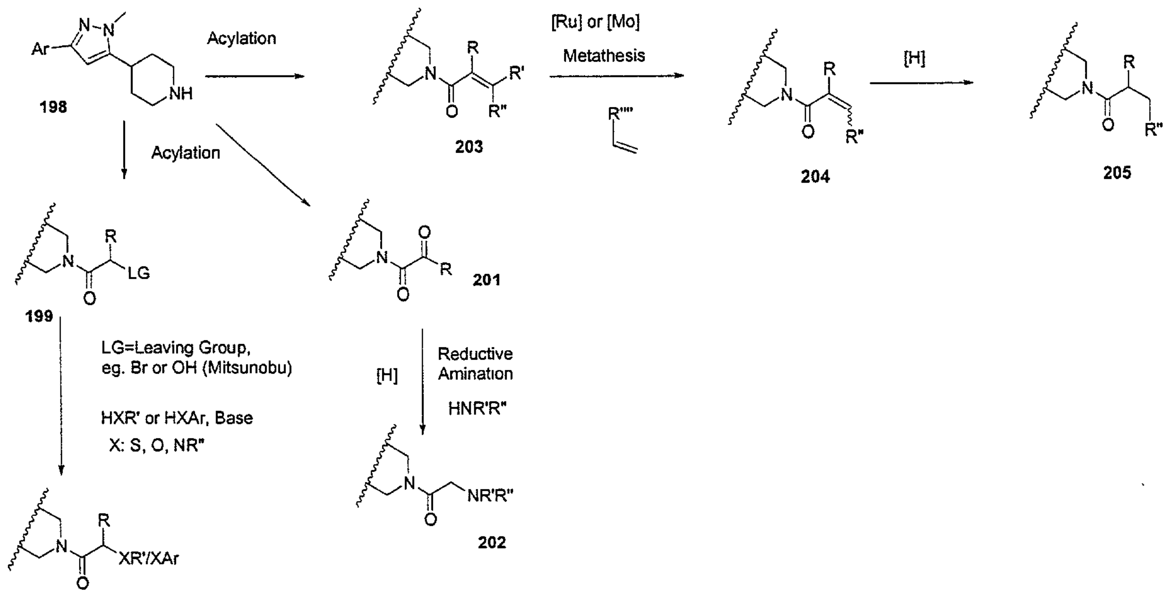
precursor since it may be used in various palladium-catalyzed transformations to provide further intermediates/analogues such as the carboxylic acid **182** (via palladium-catalyzed carbonylation using carbon monoxide, see, for example, *Tetrahedron Lett.* **1992**, *33*, 3939-3942), biaryls, arylvinyls, and arylalkyls **183** and **184** (via Suzuki/Stille/Heck couplings). Compound **181** may also be used to prepare aryl sulfides such as **185** and **186** (see, for example, Nan Zheng et al., *J. Org. Chem.* **1998**, *63*, 9606-9607.). Further, triflated phenols such as **181** can be used as substrates for the preparation of substituted aromatic amines such as **187** using conditions developed by, for example, Buchwald (see, for example, *J. Org. Chem.* **2000**, *65*, 1158-1174. and references therein). In addition, triflate **181** may be converted to the corresponding aldehyde, **188**, using palladium catalysis under a carbon monoxide atmosphere in the presence of a reducing agent such as a trialkylsilane.

Carboxylic acid **182** can be used to make amides such as **189** and esters such as **190**. In addition, compound **182** can be subjected to a Curtius rearrangement to provide the aniline **191** which in turn may be converted to amides, ureas, and carbamates **192**.

The aromatic aldehyde **188** can be reduced to the corresponding benzylic alcohol **193** or subjected to reductive amination to furnish benzylic amines such as **194**. The alcohol **193** may be alkylated or converted to esters to yield compounds such as **195**. It may also be converted to an alkylating agent such **196** (for example, a benzylic bromide) which in turn can be used to provide benzylic thiols such as **197**.

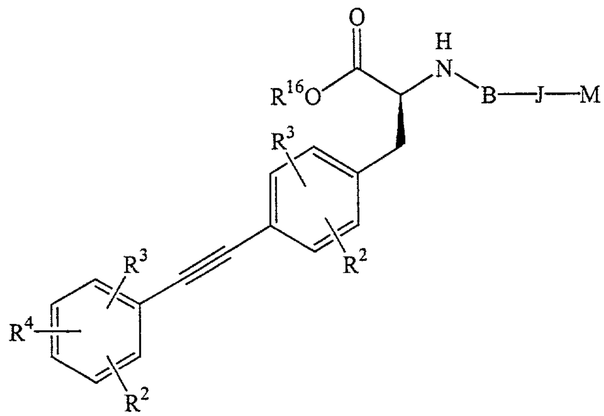
Elaboration of the -B-J-M portion of the molecule may be accomplished by the methods generally described in Examples 1 through 76, and as illustrated in Scheme II

Scheme III



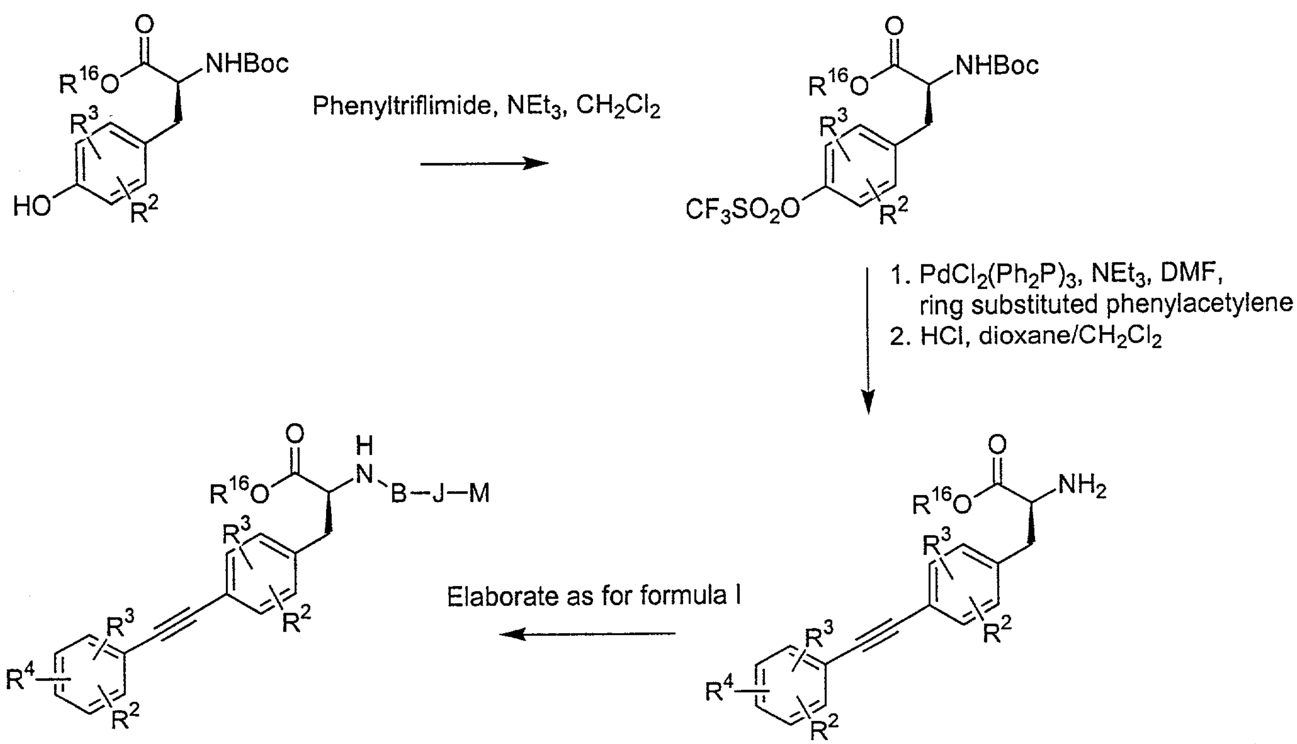
Referring to Scheme III above, the piperidine derivative **198** (e.g. **25**) can be reacted with acylating agents containing a leaving group (e.g., bromine) at, for example, the ω-position to provide alkylating agents such as **199**. These alkylating agents may be reacted further with nucleophiles, such as amines or alcohols, to provide compounds (**200**) with a linkage to a guanidine-like fragment or a guanidine mimetic. Compound **198** may also be acylated to provide α-diketo derivatives such as **201**. These compounds may be used in reductive aminations to provide additional linked guanidine type compounds **202**. Alternatively, **198** can be acylated to provide acrylic derivatives such as **203**, which can be used in a cross-metathesis transformation (for a review and leading references, see: *Tetrahedron* **1998**, *39*, 2805), yielding compounds **204** which may be further manipulated (reduced) to analogues such as **205**.

The preparation of compounds of formula I′



I′

is accomplished generally by the method of Tilley et al., *J. Am. Chem. Soc.* **1997**, *119*, 7589-7590 and its supplemental materials, and Tilley et al., *J. Org. Chem.* **1995**, *55*, 906-910, i.e.:



An optionally ring-substituted tyrosine is protected at the amine nitrogen, then treated with phenyltriflimide to give the 4-trifluoromethanesulfonyloxy compound. This is reacted with an optionally ring-substituted phenylacetylene in the presence of a palladium catalyst, and the amine deprotected with acid. Elaboration of the amine to form the compounds of formula I′ proceeds in the same manner as for

the compounds of formula I. It is assumed in this scheme that R^{16} is not hydrogen or acetylamino-C_1-C_{12}alkyl; if R^{16} is desired to be either of these, then the scheme may be carried through using as R^{16} a protecting group which is removed by hydrolysis at the appropriate point in the synthesis to give the compound of formula I′ where R^{16} is hydrogen, and optionally then treated to give the appropriate N-alkylcarbamate.

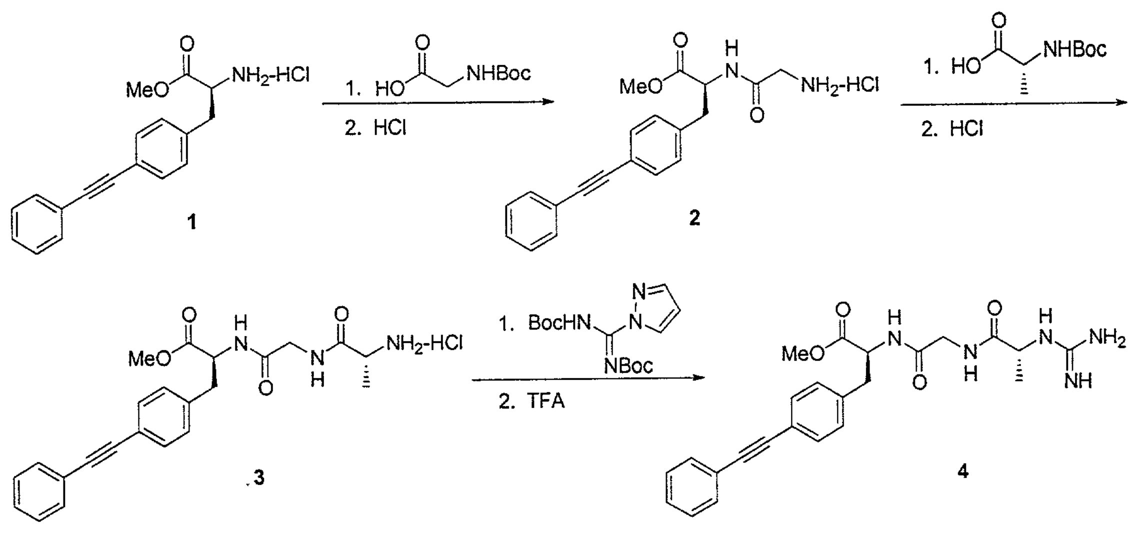
The syntheses are illustrated generally in the following Examples 1-76.

Other methods of synthesis are also usable, and a person of ordinary skill in the art, having regard to that skill, this disclosure, and the references cited herein, will be able to prepare desired compounds of this invention without undue experimentation.

Examples

The following non-limiting examples illustrate the invention. All commercially available materials were used as received. All synthesized compounds were characterized by ^{1}H NMR (Bruker DMX 400 MHz spectrometer) and/or electrospray mass-spectroscopy (ES (+) MS, Hewlett-Packard Series 1100 MSD). Abbreviations for reagents and methods are those conventional in the art.

Example 1



Amine HCl salt **1** was prepared according to the method of Tilley et al., *J. Am. Chem. Soc.* **1997**, *119*, 7589-7590 and its supplemental materials, and Tilley et al., *J. Org. Chem.* **1995**, *55*, 906-910.

a) To a solution of **1** (0.25 g, 0.8 mmol) in dichloromethane (6 mL) was added *N*-Boc-glycine (0.18 g, 1.0 mmol), EDC (0.19 g, 1.0 mmol), HOBt (0.15 g, 1.0 mmol), and triethylamine (0.28 mL, 2.0 mmol). The mixture was stirred overnight and then was partitioned between dichloromethane and water. The aqueous layer was washed with dichloromethane (2×); the organic layer was washed with 1M HCl and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to provide the desired amide.

The crude residue was dissolved in HCl/dioxane (4.0N, 5 mL), stirred for 45 minutes, and then concentrated *in vacuo* to provide **2**.

b) To a solution of **2** (0.1 g, 0.27 mmol) in dichloromethane (2 mL) was added *N*-Boc-D-alanine (66 mg, 0.35 mmol), EDC (67 mg, 0.35 mmol), HOBt (53 mg, 0.35 mmol), and triethylamine (0.1 mL, 0.7 mmol). The mixture was stirred overnight and then was partitioned between dichloromethane and

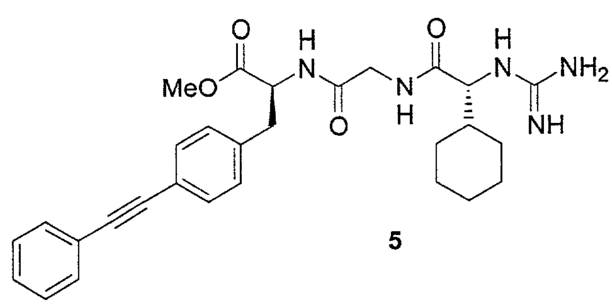
water. The aqueous layer was washed with dichloromethane; the organic layer was washed with 1M HCl and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to provide the desired amide.

The crude residue was dissolved in HCl/dioxane (4.0N, 5 mL), stirred for 30 minutes, and then concentrated *in vacuo* to provide **3** (110 mg).

c) To a solution of **3** (75 mg, 0.17 mmol) in MeOH (2.5 mL) was added (*tert-b*utoxycarbonylimino-pyrazol-1-yl-methyl)-carbamic acid *tert*-butyl ester (77 mg, 0.25 mmol) and triethylamine (45 µL, 0.32 mmol). The mixture was stirred overnight and then was partitioned between dichloromethane and water. The aqueous layer was washed with dichloromethane (2×); the organic layer was washed with 1M HCl, dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

The residue was dissolved in dichloromethane (1.5 mL) and TFA (1.5 mL) was added. The solution was stirred for 2 h and then concentrated to an oil. Purification of the material by RP HPLC to provide **4** as a white solid. ES (+) MS m/e = 450 (M+1).

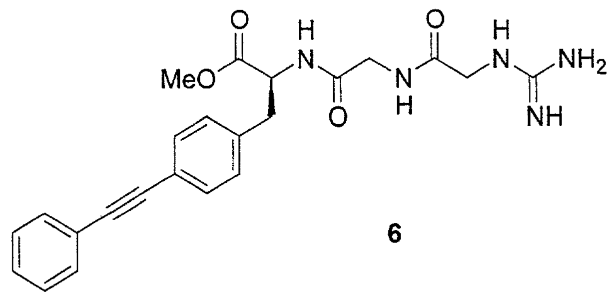
Example 2



5

Title compound **5** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-cyclohexylglycine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 518 (M+1).

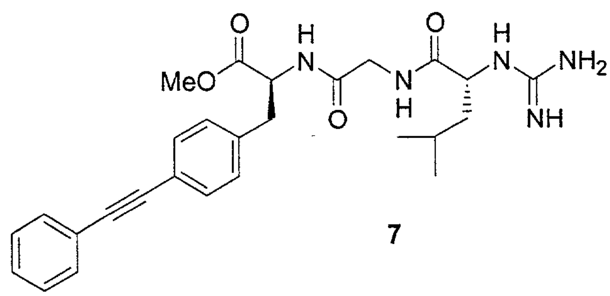
Example 3



6

Title compound **6** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-glycine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 436 (M+1).

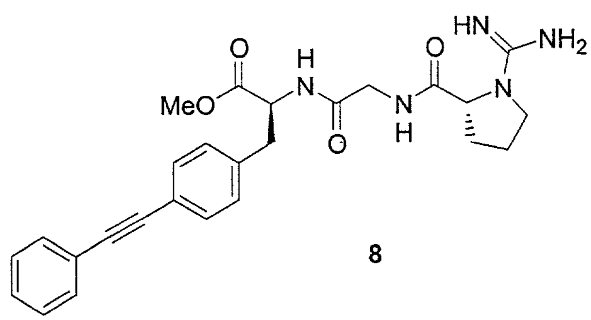
Example 4



7

Title compound **7** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-leucine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 492 (M+1).

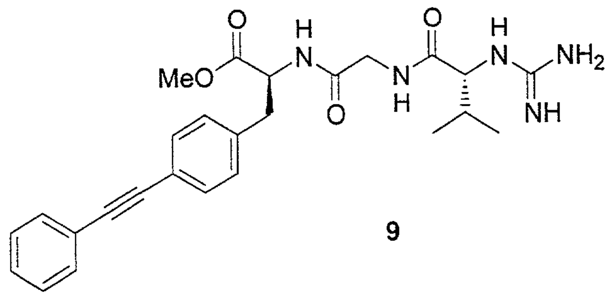
Example 5



8

Title compound **8** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-proline as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 476 (M+1).

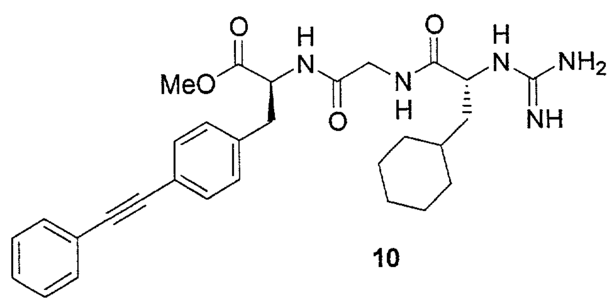
Example 6



9

Title compound **9** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-valine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 478 (M+1).

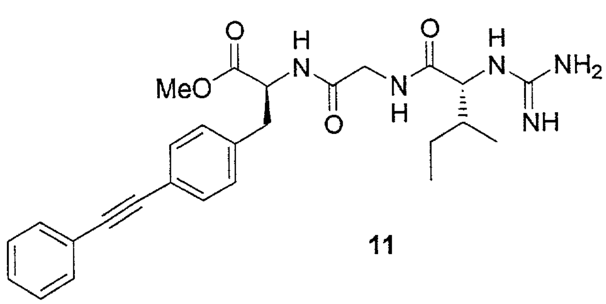
Example 7



10

Title compound **10** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-cyclohexylalanine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 532 (M+1).

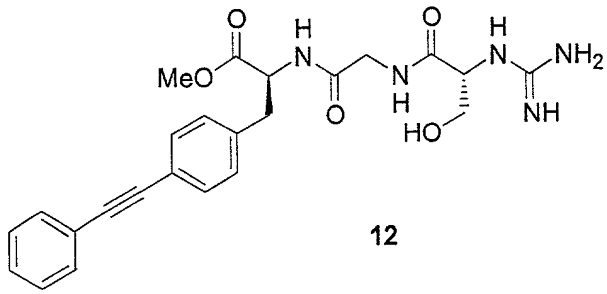
Example 8



11

Title compound **11** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-isoleucine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 492 (M+1).

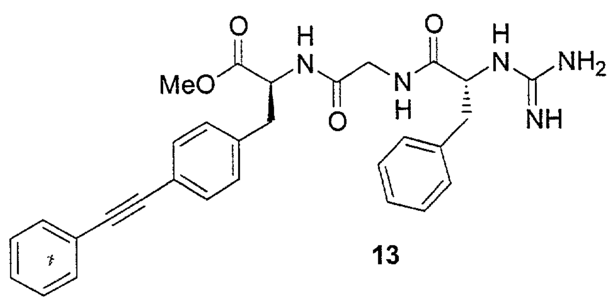
Example 9



12

Title compound **12** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-serine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 466 (M+1).

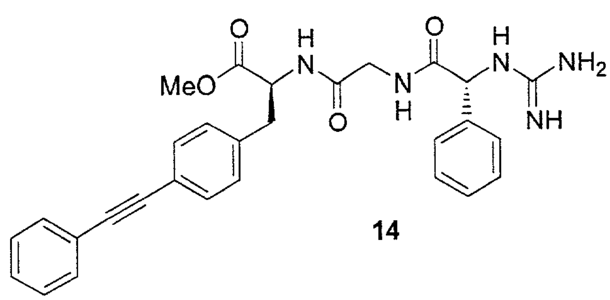
Example 10



13

Title compound **13** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-phenylalanine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 526 (M+1).

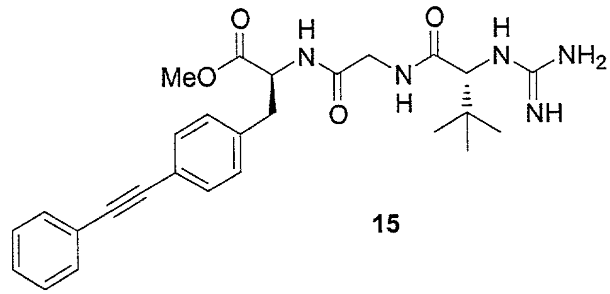
Example 11



14

Title compound **14** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-phenylglycine as a reagent instead of *N*-Boc-D-alanine. ES (+) MS m/e = 512 (M+1).

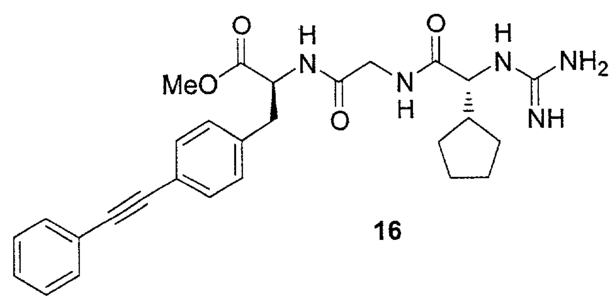
Example 12



15

Title compound **15** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-D-*tert*-butylglycine as a reagent instead of *N*-Boc-glycine. ES (+) MS m/e = 492 (M+1).

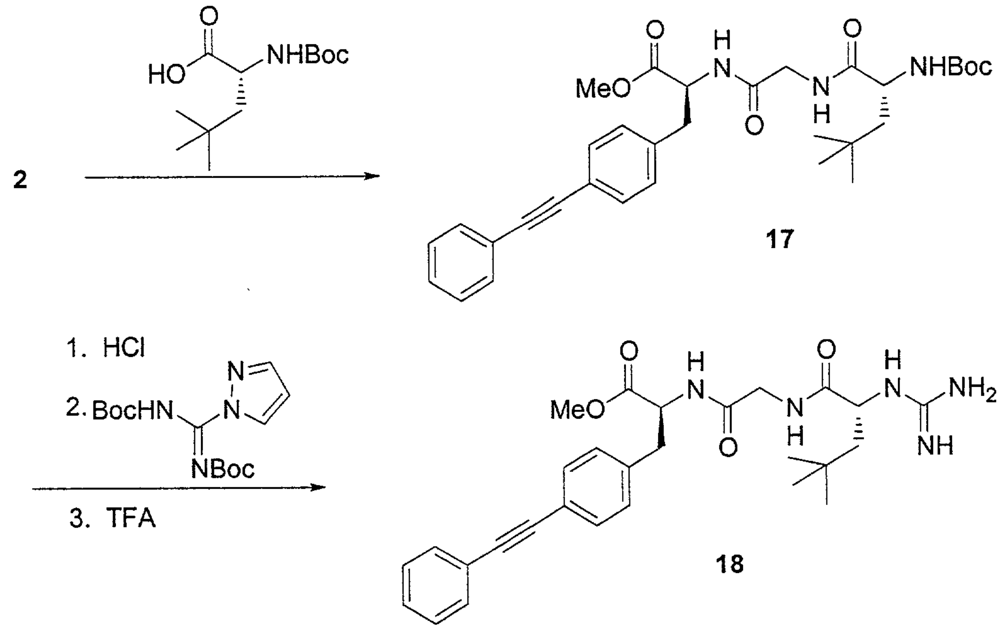
Example 13



16

Title compound **16** was prepared according to the procedure of Example 1a-c except for using *N*-Boc-cyclopentylglycine as a reagent instead of *N*-Boc-glycine. ES (+) MS m/e = 504 (M+1).

Example 14



a) To a solution of **2** (0.48 g, 1.3 mmol) in dichloromethane (8 mL) was added *N*-Boc-D-*t*-butylalanine (0.39 g, 1.6 mmol), EDC (0.3 g, 1.6 mmol), HOBt (0.25 g, 1.6 mmol), and triethylamine (0.45 mL, 3.2 mmol). The mixture was stirred overnight and then was partitioned between dichloromethane and water. The aqueous layer was washed with dichloromethane; the organic layer was washed with 1M HCl and sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to provide **17** (0.67 g).

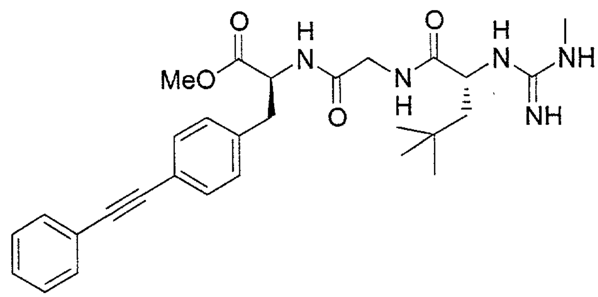
b) Amide **17** (~0.2 mmol) was dissolved in HCl/dioxane (4.0N, 5 mL), stirred for 2 h, and then concentrated *in vacuo*.

To the crude HCl salt in MeOH (1 mL) was added (*tert*-butoxycarbonylimino-pyrazol-1-yl-methyl)-carbamic acid *tert*-butyl ester (93 mg, 0.3 mmol) and triethylamine (84 μL, 0.6 mmol). The reaction was stirred for 40 h and then concentrated *in vacuo*.

The dry residue was dissolved in dichloromethane (1.5 mL) and TFA (1.5 mL), stirred 3 h, and then concentrated to dryness. The material was purified by RP HPLC to provide **18** as a white solid.

ES (+) MS m/e = 506 (M+1).

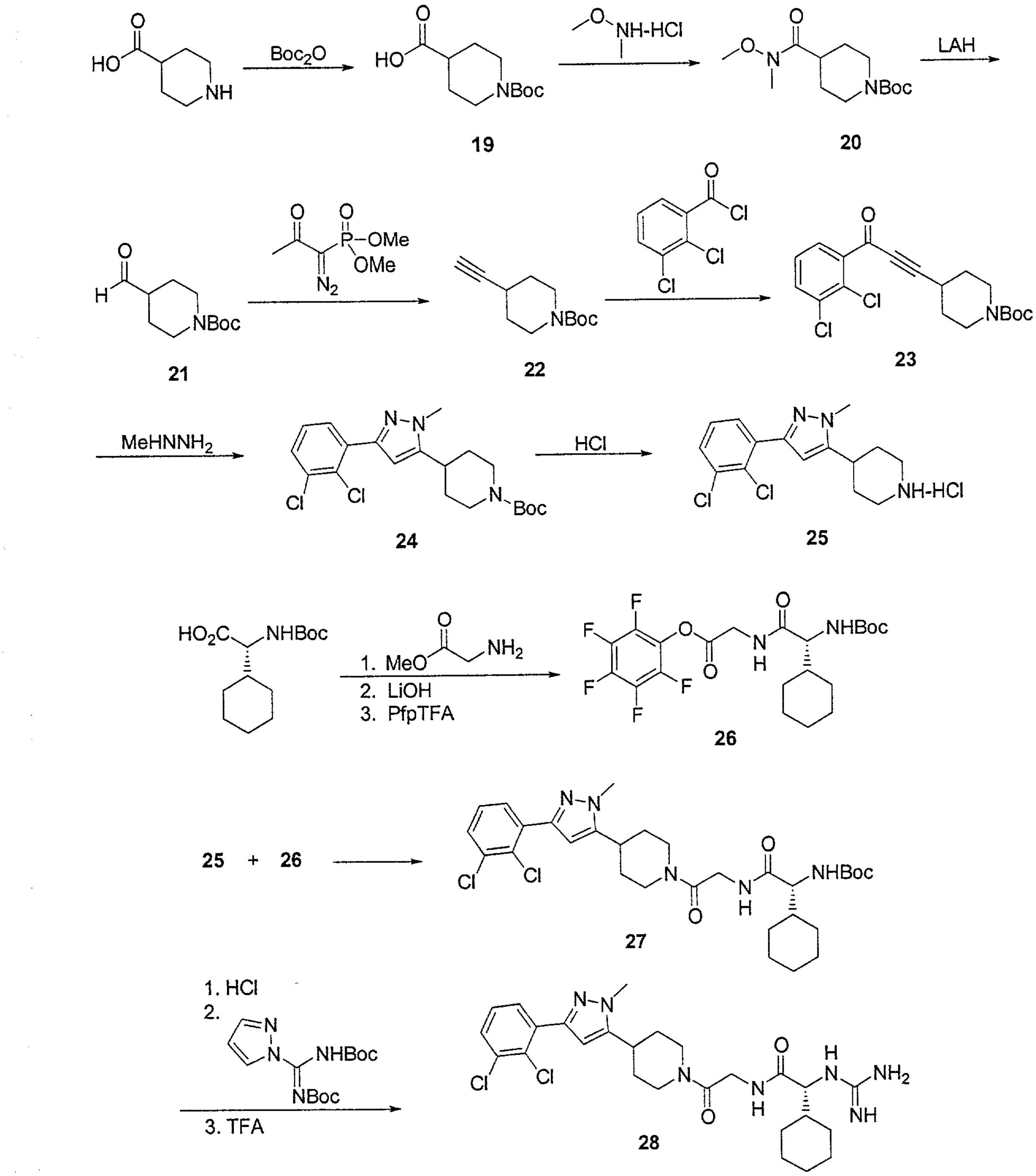
Example 15



The title compound was prepared according to the procedure of Example 14 except for using (*tert*-butoxycarbonylimino-pyrazol-1-yl-methyl)-methylcarbamic acid *tert*-butyl ester as a reagent instead of (*tert*-butoxycarbonylimino-pyrazol-1-yl-methyl)-carbamic acid *tert*-butyl ester.

ES (+) MS m/e = 520 (M+1).

Example 16



a) To a heterogeneous solution of isonipecotic acid (45.0 g, 0.35 mol), NaOH (41.8 g, 1.05 mol) in THF (350 mL) and water (650 mL) was added di-*tert*-butyl dicarbonate (91.3 g, 0.42 mol). The mixture was stirred overnight at room temperature and then diluted with ethyl acetate (500 mL). The resulting

biphasic solution was separated and the basic aqueous layer was acidified to pH 2-3 by slow addition of concentrated HCl. The aqueous layer was washed with ethyl acetate (3 × 500 mL); the combined organic layer was dried over $MgSO_4$ and then concentrated under reduced pressure to afford **19** (76.9 g, 96%). ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.59-1.64 (m, 2H), 1.89-1.92 (m, 2H), 2.46-2.48 (m, 1H), 2.82-2.88 (m, 2H), 4.01 (m, 2H).

b) To a solution containing **19** (76.9 g, 0.34 mol), HOBt (54.4 g, 0.40 mol), EDC (77.1 g, 0.40 mol), and triethylamine (160 mL, 1.14 mol) in dichloromethane (700 mL) was added *N,O*-dimethylhydroxylamine hydrochloride (39.3 g, 0.40 mol). The resulting mixture was stirred overnight at room temperature. The solution was partitioned between water and dichloromethane; the aqueous layer was washed with ethyl acetate (2 × 500 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to afford crude **20** (79.5 g, 87%). ES (+) MS m/e = 217 (M-55).

c) To a solution of **20** (1.0 g, 3.7 mmol) in THF (8.0 mL) at –78°C was added dropwise lithium aluminum hydride (1.0M in THF, 4.0 mL, 4.0 mmol). Upon complete addition, the reaction mixture was stirred for 15 minutes at –78°C. The reaction was quenched by slow addition of isopropanol followed by aqueous 1.0M HCl (50 mL). The aqueous layer was washed with ethyl acetate (3 × 50 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford crude **21** (0.8 g, 99%). ES (+) MS m/e = 158 (M-55).

d) To a heterogeneous solution of **21** (23.7 g, 0.11 mol) and potassium carbonate (30.7 g, 0.22 mol) in MeOH (1.0 L) was added dropwise dimethyl-1-diazo-2-oxopropylphosphonate (21.3 g, 0.11 mol) [Muller et al., *Syn. Lett.*, **1996**, 521] in MeOH (100 mL). The resulting mixture was stirred for 3 hours and concentrated under reduced pressure. The residue was diluted with diethyl ether (500 mL) and 5% aqueous $NaHCO_3$ (700 mL). The layers were separated and the aqueous layer was washed with diethyl ether (2 × 500 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford crude **22** (22.4 g, 96%). ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.57-1.60 (m, 2H), 1.75-1.77 (m, 2H), 2.09 (s, 1H), 2.57-2.58 (m, 1H), 3.15-3.20 (m, 2H), 3.67 (m, 2H).

e) To a solution of **22** (22.4 g, 107 mmol), 2,3-dichlorobenzoyl chloride (22.4 g, 107 mmol), copper(I) iodide (1.0 g, 5.3 mmol), and triethylamine (15 mL, 107 mmol) in toluene (500 mL) was added dichlorobis(triphenylphosphine)palladium(II) (3.75 g, 5.3 mmol). The resulting mixture was stirred overnight and then diluted with saturated sodium bicarbonate (500 mL) and ethyl acetate

(500 mL). The aqueous layer was washed with ethyl acetate (500 mL); the organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2: 25 to 50% ethyl acetate in hexanes) to afford **23** (31.9 g, 78%). ES (+) MS m/e = 326 (M-55).

f) To a solution of **23** (31.9 g, 83.4 mmol) in ethanol (160 mL) at 0°C was added methylhydrazine (8.9 mL, 167 mmol). The resulting mixture was stirred for 15 minutes at 0°C and then concentrated under reduced pressure. Purification of the residue by flash chromatography (SiO_2: 25 to 50% ethyl acetate in hexanes) afforded **24** (16.0 g, 46%) as a pure regioisomer. Concentration of mixed fractions provided additional **24** (16.0 g, 46%) as a 9:1 mixture of regioisomers. ES (+) MS m/e = 410 (M+1).

g) To a solution of **24** (16.0 g, 38.4 mmol) in dioxane (100 mL) was added HCl/dioxane (4.0N, 100 mL). The reaction mixture was stirred at room temperature for 1 hour and then was concentrated under reduced pressure to afford **25** (14.4 g, 99%). ES (+) MS m/e = 310 (M+1).

h) To *N*-Boc-D-cyclohexylglycine (10.0 g, 38.9 mmol) in dichloromethane (100 mL) was added EDC (8.2 g, 42.8 mmol), HOBt monohydrate (6.5 g, 42.8 mmol) and triethylamine (11.9 mL, 85.5 mmol). Glycine methyl ester hydrochloride (5.8 g, 46.6 mmol) was added and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with dichloromethane (60 mL) and partitioned with water (70 mL). The organic layer was separated, and the aqueous layer was extracted with dichloromethane (2 × 60 mL). The combined organic layer was washed with 1M HCl (60 mL), saturated $NaHCO_3$ (60 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the desired amide (12.0 g, 94%).

To the amide (12.0 g, 36.7 mmol) in THF/water (3:1, 150 mL) was added lithium hydroxide (2.0 g, 83.5 mmol). The reaction mixture was stirred at room temperature overnight and then acidified using 1M HCl (100 mL). The mixture was extracted with ethyl acetate (3 × 100 mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to yield the desired carboxylic acid (9.7 g, 84%).

To the acid (9.7 g, 30.9 mmol) in THF (120 mL) was added pyridine (2.8 mL, 34.0 mmol) and pentafluorophenyl trifluoroacetate (5.8 mL, 34.0 mmol). The reaction mixture was stirred at room temperature for 2 hours and then the solvent was removed under reduced pressure. The resulting residue was dissolved in ethyl acetate (100 mL) and was washed with 1M HCl (70 mL) and saturated $NaHCO_3$

(70 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to afford **26** (13.5 g, 91%). ES (+) MS m/e = 425 (M-55).

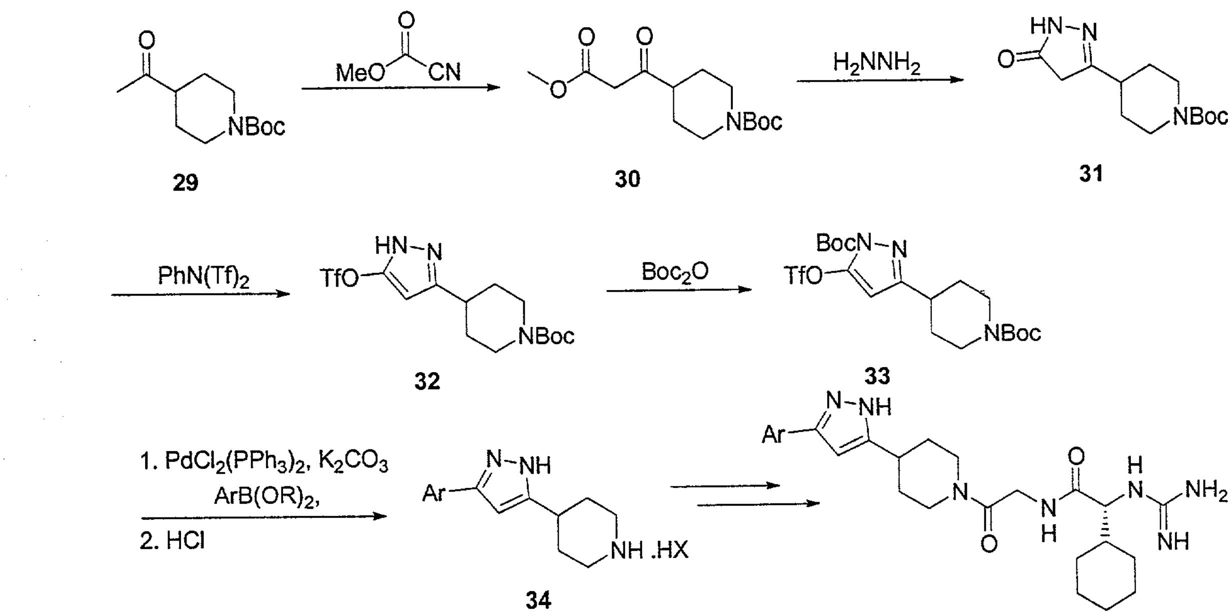
To amine hydrochloride salt **25** (19 mg, 0.06 mmol) in dichloromethane (1 mL) with triethylamine (19 µL, 0.13 mmol) was added ester **26** (32 mg, 0.07 mmol). The reaction was stirred at room temperature for 3 h and then the solvent was removed under reduced pressure to yield **27** which was used without purification. ES (+) MS m/e = 606 (M+1).

A solution of **27** (34 mg, 0.06 mmol) in HCl/dioxane (4N, 1 mL) was stirred at room temperature for 30 min. The solvent was removed under reduced pressure to provide the desired amine as the hydrochloride salt which was used without purification.

To the amine hydrochloride salt (30 mg, 0.06 mmol) in MeOH (1 mL) with triethylamine (23 µL, 0.17 mmol) was added *N,N'*-bis-Boc-1-guanylpyrazole (26 mg, 0.08 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure to yield the protected guanidine which was used without purification.

The protected guanidine (42 mg, 0.06 mmol) was dissolved in TFA/dichloromethane (1:1, 1 mL) and stirred at room temperature for 3h. The solvent was removed under reduced pressure to afford the crude guanidine as the trifluoroacetate salt. The crude material was purified by RP HPLC to provide **28**. ES (+) MS m/e = 548 (M+1).

Example 17



a) To a solution of **29** (25 g, 110 mmol) in THF (435 mL) at -78°C was added dropwise lithium bis(trimethylsilyl)amide (1.0M in THF, 220 mL, 220 mmol). To the resulting mixture was added methyl cyanoformate (15.7 mL, 198 mmol). After 30 minutes at -78°C, the reaction was warmed to room temperature, diluted with diethyl ether, and then quenched with water. The mixture was extracted with water, 0.5N aqueous HCl, and brine. The organic layer was dried over Na_2SO_4 and concentrated to yield **30** (33.4 g) as a colorless oil. A pure sample was obtained by flash column chromatography (SiO_2: gradient 0 to 40% ethyl acetate in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 (br s, 2 H), 3.73 (s, 3 H), 3.50 (s, 2 H), 2.77 (m, 2 H), 2.60 (m, 1 H), 1.83 (m, 2 H), 1.57 (m, 2 H), 1.44 (s, 9 H); TLC (SiO_2: 20% ethyl acetate in hexane): $R_f = 0.13$, TLC (SiO_2; 50% ethyl acetate in hexane): $R_f = 0.34$; ES (+) MS m/e = 286 (M+1).

b) A mixture of the crude product **30** (32.8 g) obtained in the previous step and hydrazine hydrate (5.58 mL, 115 mmol) in ethanol (120 mL) was heated at 70°C until TLC indicated complete consumption of the starting material (~ 1h). The reaction was cooled to ambient temperature and the resulting precipitate (11.5 g of **31**) was collected by filtration. The filtrate was concentrated, re-dissolved in hot ethanol, and allowed to reach room temperature. After standing for several hours a second crop of **31** (4.3 g) was collected and when combined provided **31** (15.8 g, 54% from **29**) as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 4.11 (m, 2 H), 3.30 (m, 1 H), 2.86 (br s, 2 H), 2.73 (m, 2 H), 1.88

(m, 2 H), 1.53 (m, 2 H), 1.46 (s, 9 H); TLC (SiO_2: 10% methanol in dichloromethane): $R_f = 0.27$; ES (+) MS m/e = 268 (M+1).

c) A solution of **31** (11.5 g, 43.0 mmol) and *N*-phenyltrifluoromethanesulfonimide (19.9 g, 55.9 mmol) in anhydrous dichloromethane (150 mL) under nitrogen at 0°C was treated with triethylamine (60 mL, 430 mmol). The mixture was allowed to reach ambient temperature. After 1 h, HPLC indicated complete consumption of **31** and the solvent was removed under reduced pressure. Purification of the crude residue by flash column chromatography (SiO_2: gradient 0 to 40% ethyl acetate in hexane) yielded **32** (14.8 g, 86%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 5.95 (s, 1 H), 4.13 (br s, 2 H), 2.82 (m, 3 H), 1.94 (m, 2 H), 1.61 (m, 2 H), 1.46 (s, 9 H); TLC (SiO_2; 50% ethyl acetate in hexane): $R_f = 0.45$; ES (+) MS m/e = 400 (M+1).

d) To a solution of **32** (14.8 g, 36.9 mmol) in dichloromethane (35 mL) was added a mixture of di-*tert*-butyl dicarbonate (12.1 g, 55.6 mmol), DMAP (0.45 g, 3.71 mmol), and triethylamine (75 mL) in dichloromethane (70 mL). After 1 h, HPLC indicated complete conversion of the starting material. The solvent was removed under reduced pressure and the crude residue was purified by flash column chromatography (SiO_2: gradient 0 to 10% ethyl acetate in hexane) to yield **33** (16.9 g, 91%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 6.08 (s, 1 H), 4.23 (br s, 2 H), 3.47 (m, 1 H), 2.81 (m, 2 H), 2.00 (m, 2 H), 1.64 (s, 9 H) , 1.49 (m, 2 H) , 1.47 (s, 9 H); TLC (SiO_2: 20% ethyl acetate in hexane): $R_f = 0.36$; ES (+) MS m/e = 344 (M-156).

e) Suzuki couplings with **33**.

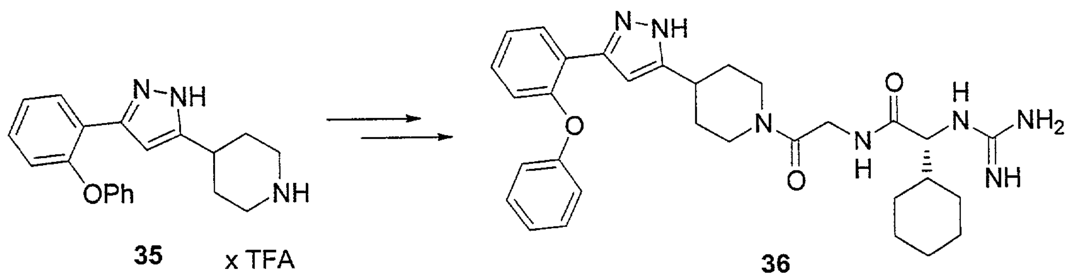
General Procedure A: In a typical procedure aqueous potassium carbonate (2M, 200 mol%) was added to a solution of **33** (1M, 100 mol%, 0.2-0.4 mmol) and the arylboronic acid (110 mol%) in DMF/dioxane (2.5:1). The resulting solution was heated to 110°C followed by addition of $PdCl_2(PPh_3)_2$ (10 mol%). The reaction was monitored by HPLC and allowed to cool to room temperature when **33** had been completely consumed. The mixture was filtered through a plug of Celite filter aid and concentrated. The residue was dissolved in dichloromethane (0.5 mL) and treated with HCl/dioxanes (4N, 4 mL) for 1h. After removal of the solvent under reduced pressure, the crude residue was purified by RP HPLC to yield the corresponding 4-(5-aryl-pyrazol-3-yl)-piperidine trifluoroacetic acid salt **34**.

General Procedure B: The Suzuki coupling was carried out as described in the general procedure A. The crude product was first purified by RP HPLC and then dissolved in dichloromethane (0.5 mL) and

treated with HCl/dioxanes (4N, 4 mL) for 1h. Removal of the solvent under reduced pressure yielded the corresponding 4-(5-aryl-pyrazol-3-yl)-piperidine hydrochloride salt **34**.

General Procedure C: The Suzuki coupling was carried out as described in the general procedure A. The crude product was first purified by flash column chromatography and then dissolved in dichloromethane (0.5 mL) and treated with HCl/dioxane (4N, 4 mL) for 1h. Removal of the solvent under reduced pressure yielded the corresponding 4-(5-aryl-pyrazol-3-yl)-piperidine hydrochloride salt **34**.

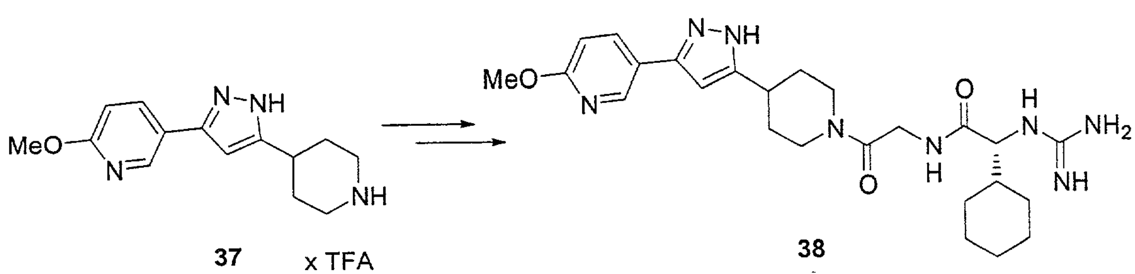
Example 18



General Procedure A of Example 17e above was followed using 100 mg (0.20 mmol) of **33** and 4-phenoxyboronic acid to provide **35** (56 mg, 64%). ES (+) MS m/e = 320 (M+1).

Title compound **36** was prepared according to the procedure of Example 16i,j except for using **35** as a reagent instead of **25**. ES (+) MS m/e = 558 (M+1).

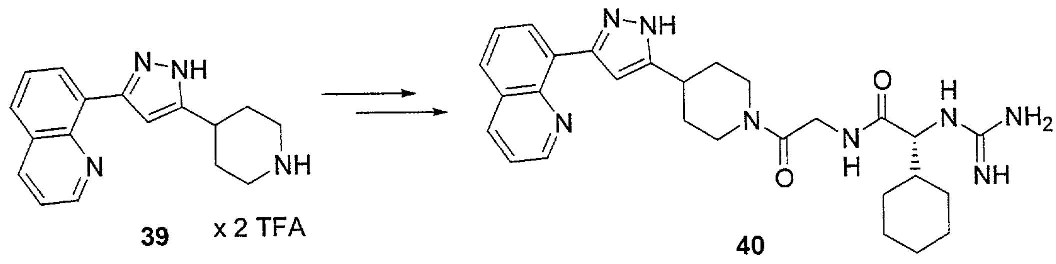
Example 19



General Procedure A of Example 17e above was followed using 200 mg (0.40 mmol) of **33** and 2-methoxy-5-pyridineboronic acid to provide **37** (45 mg, 30%). ES (+) MS m/e = 259 (M+1).

Title compound **38** was prepared according to the procedure of Example 16i,j except for using **37** as a reagent instead of **25**. ES (+) MS m/e = 497 (M+1).

Example 20

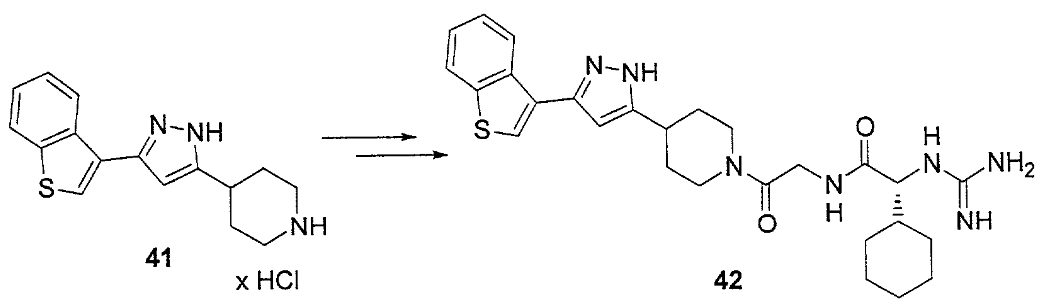


39 x 2 TFA

40

General Procedure A of Example 17e above was followed using 150 mg (0.30 mmol) of **33** and 8-quinolineboronic acid to provide **39** (48 mg, 32%). ES (+) MS m/e = 279 (M+1). Title compound **40** was prepared according to the procedure of Example 16i,j except for using **39** as a reagent instead of **25**. ES (+) MS m/e = 517 (M+1).

Example 21

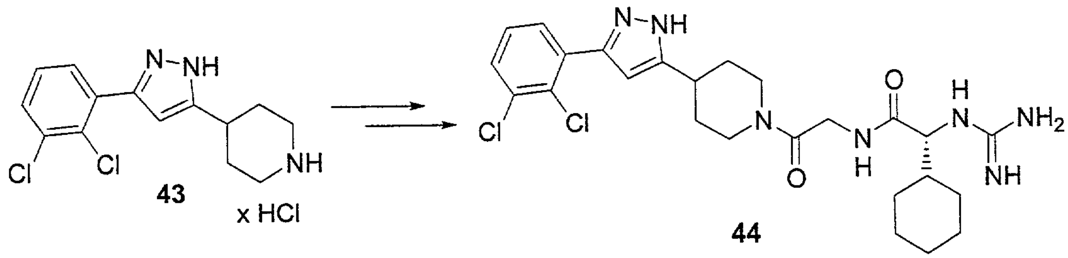


41 x HCl

42

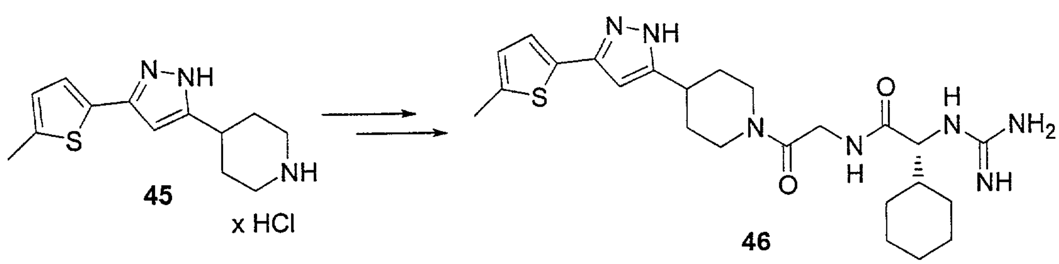
General Procedure B of Example 17e above was followed using 200 mg (0.40 mmol) of **33** and benzothiaphene-3-boronic acid to provide **41** (55 mg, 43%). ES (+) MS m/e = 284 (M+1). Title compound **42** was prepared according to the procedure of Example 16i,j except for using **41** as a reagent instead of **25**. ES (+) MS m/e = 522 (M+1).

Example 22



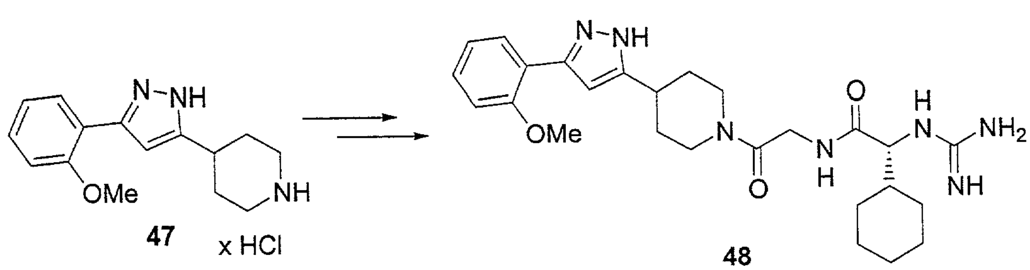
General Procedure B of Example 17e above was followed using 200 mg (0.40 mmol) of **33** and (2,3-dichlorophenyl)boronic acid to provide **43** (31 mg, 23%). ES (+) MS m/e = 296 (M+1). Title compound **44** was prepared according to the procedure of Example 16i,j except for using **43** as a reagent instead of **25**. ES (+) MS m/e = 534 (M+1).

Example 23



General Procedure B of Example 17e above was followed using 200 mg (0.40 mmol) of **33** and 5-methylthiophene-2-boronic acid to provide **45** (45 mg, 40%). ES (+) MS m/e = 248 (M+1). Title compound **46** was prepared according to the procedure of Example 16i,j except for using **45** as a reagent instead of **25**. ES (+) MS m/e = 486 (M+1).

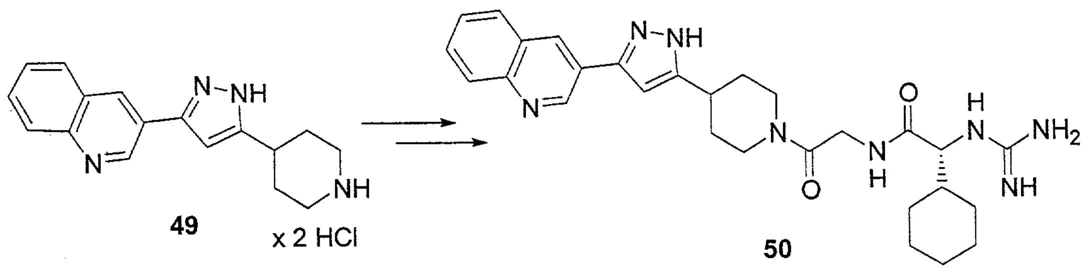
Example 24



General Procedure C of Example 17e above was followed using 100 mg (0.20 mmol) of **33** and (2-methoxyphenyl)boronic acid to provide **47** (40 mg, 69%). ES (+) MS m/e = 258 (M+1).

Title compound 48 was prepared according to the procedure of Example 16i,j except for using 47 as a reagent instead of 25. ES (+) MS m/e = 496 (M+1).

Example 25



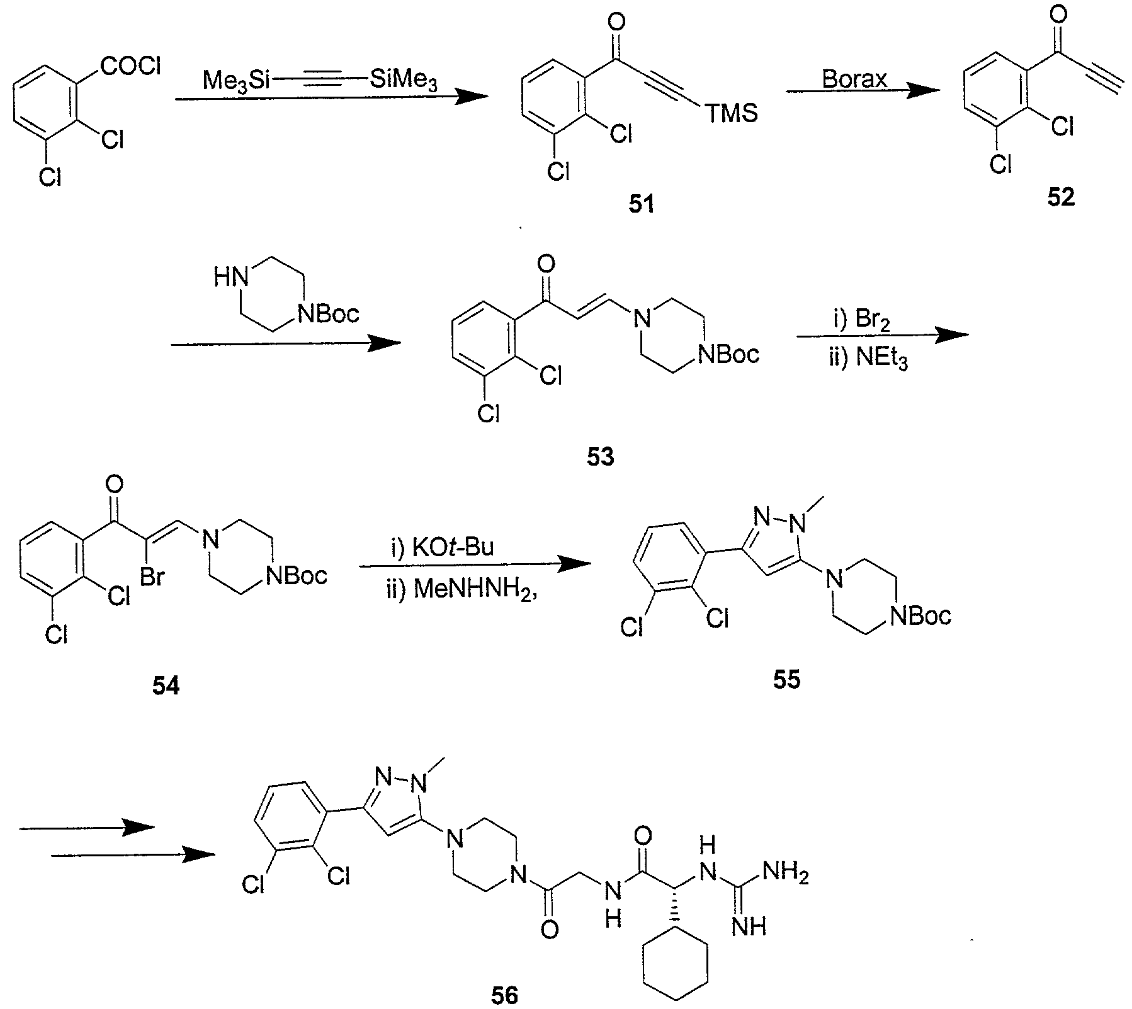
49

50

General Procedure C of Example 17e above was followed using 100 mg (0.20 mmol) of **33** and 3-quinolineboronic acid to provide **49** (61 mg, 87%). ES (+) MS m/e = 279 (M+1).

Title compound **50** was prepared according to the procedure of Example 16i,j except for using **49** as a reagent instead of **25**. ES (+) MS m/e = 517 (M+1).

Example 26



a) To a 0°C solution of 2,3-dichloro-benzoyl chloride (10.0 g, 47.7 mmol) and bis(trimethylsilyl)acetylene (13.0 mL, 57.3 mmol) in anhydrous dichloromethane (240 mL) was added aluminum chloride (7.40 g, 55.8 mmol) portionwise. The resulting mixture was stirred under nitrogen at 0°C for 1 h. The reaction mixture was poured into ice-water, the phases were separated, and the aqueous layer was extracted with dichloromethane (2×). The combined organic layer was washed with 5% aqueous $NaHCO_3$ (3×), dried (Na_2SO_4), and concentrated, avoiding heating. The residue was taken up in hexane, filtrated through a plug of silica gel, and concentrated, avoiding heating, to give **51** (11.9 g, 92%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, 1 H, J = 7.8 Hz), 7.64 (d, 1 H, J = 8.0 Hz), 7.33 (app t, 1 H, J = 7.9 Hz), 0.29 (s, 9 H); TLC (SiO_2: 20% ethyl acetate in hexane): R_f = 0.46; ES (+) MS m/e = 271 (M+1).

b) To a mixture of **51** (11.9 g, 43.9 mmol) in THF/H_2O/MeOH (5:1:3, 90 mL) was added borax (353 mg, 1.76 mmol). The reaction was stirred at room temperature and closely monitored by TLC (SiO_2: 20% ethyl acetate in hexane). When almost all of **51** had been consumed (~15 minutes), ethyl acetate was added and the mixture was extracted with 1% aqueous citric acid. The phases were separated and the aqueous layer was extracted with ethyl acetate (2×). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (SiO_2: gradient 0 to 3% ethyl acetate in hexane) to yield **52** (6.30 g, 71%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, 1 H, J = 7.8 Hz), 7.67 (d, 1 H, J = 8.1 Hz), 7.35 (app t, 1 H, J = 7.9 Hz), 3.51 (s, 1 H); TLC (SiO_2: 20% ethyl acetate in hexane): R_f = 0.27; ES (+) MS m/e = 199 (M+1).

c) To a mixture of **52** (300 mg, 1.51 mmol) and piperazine-1-carboxylic acid *tert*-butyl ester (337 mg, 1.81 mmol) was added anhydrous dichloromethane (4 mL). After 45 min TLC indicated complete consumption of **52**. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (SiO_2: gradient 40 to 70% ethyl acetate in hexane) to yield **53** (310 mg, 53%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, 1 H, J = 7.5 Hz), 7.22 (m, 3 H), 5.48 (d, 1 H, J = 12.3 Hz), 3.51 (m, 4 H), 3.34 (m, 4 H), 1.47 (s, 9 H); TLC (SiO_2: 50% ethyl acetate in hexane): R_f = 0.12; ES (+) MS m/e = 385 (M+1).

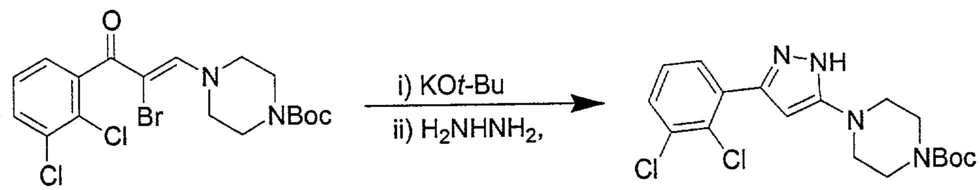
To a solution of 53 (300 mg, 0.78 mmol) in dichloromethane (0.65 mL) at 0°C under nitrogen was added bromine (42 μL, 0.82 mmol) dropwise. After 10 min, the reaction was diluted with diethyl ether (0.5 mL) and triethylamine (114 μL, 0.81 mmol) was added dropwise. After 1h at 0°C, the white precipitate was removed by filtration and the filtrate was concentrated *in vacuo* to provide 54 (359 mg, 99%) as a light yellow foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, 1 H, J = 7.9 Hz), 7.24 (m, 2 H), 7.15 (d, 1 H, J = 7.5 Hz), 3.71 (br s, 4 H), 3.51 (m, 4 H), 1.46 (s, 9 H); ES (+) MS m/e = 465 (M+1).

d) To a 0°C solution of **54** (359 mg, 0.80 mmol) in THF (anhydrous, 3 mL) under nitrogen was added potassium *tert*-butoxide (97 mg, 0.80 mmol). After 1 h the reaction was allowed to reach ambient temperature and a solution of methylhydrazine (211 μL, 3.98 mmol) in dichloromethane (1 mL) was added. The resulting mixture was heated at 50°C for 1.5h. After cooling to room temperature, diethyl ether was added and the reaction mixture was washed with water. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (SiO_2: gradient 20 to 50% ethyl acetate in hexane) to yield **55** (260 mg, 80%) as a colorless film. ^{1}H NMR (400 MHz,

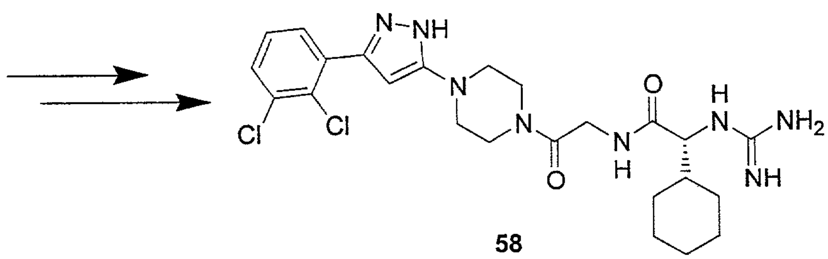
$CDCl_3$) δ 7.64 (d, 1 H, J = 7.8 Hz), 7.38 (d, 1 H, J = 7.9 Hz), 7.18 (app t, 1 H, J = 8.0 Hz), 6.31 (s, 1 H), 3.77 (s, 3 H), 3.56 (m, 4 H), 2.91 (m, 4 H), 1.46 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.0, 151.8, 147.6, 135.3, 134.1, 130.9, 129.9, 129.4, 127.6, 96.4, 80.5, 52.5, 44.0, 35.4, 28.8; The regiochemistry was determined by NOESY. TLC (SiO_2: 50% ethyl acetate in hexane): R_f = 0.44; ES (+) MS m/e = 411 (M+1).

e) Title compound **56** was prepared according to the procedure of Example 16g,i,j except for using **55** as a reagent instead of **24**. ES (+) MS m/e = 549 (M+1).

Example 27



54 **57**

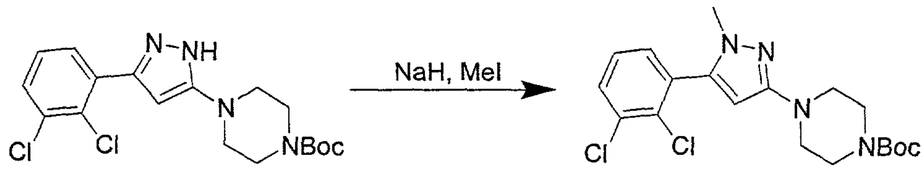


58

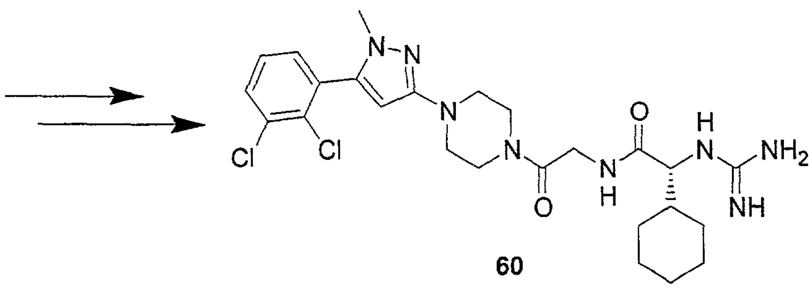
a) Pyrazole **57** was synthesized from **54** (1.00 g, 2.15 mmol) according to the procedure of Example 26e except hydrazine (1.0M in THF, 10.8 mL, 10.8 mmol) was used as a reagent and **57** was isolated as a white solid (0.25 g, 29%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, 1 H, J = 8.0 Hz), 7.39 (d, 1 H, J = 7.8 Hz), 7.24 (app t, 1 H, J = 7.9 Hz), 6.03 (s, 1 H), 3.58 (m, 4 H), 3.23 (m, 4 H), 1.48 (s, 9 H); TLC (SiO_2: 50% ethyl acetate in hexane): R_f = 0.15; ES (+) MS m/e = 341 (M-56).

b) Title compound **58** was prepared according to the procedure of Example 16g,i,j except for using **57** as a reagent instead of **24**. ES (+) MS m/e = 535 (M+1).

Example 28



57 59

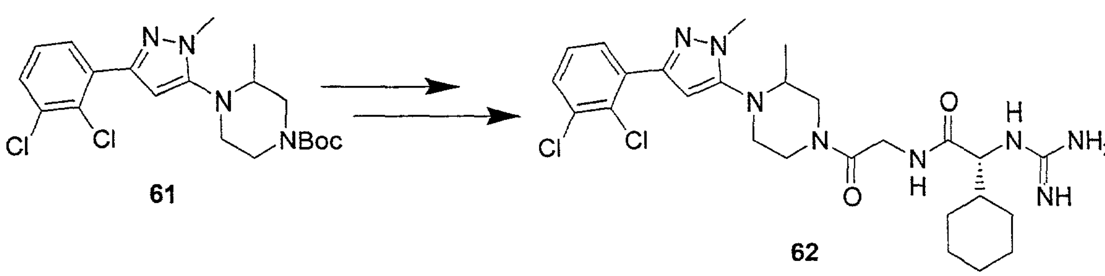


60

a) To a solution of **57** (130 mg, 0.33 mmol) in anhydrous THF (2 mL) was added sodium hydride (60% in mineral oil, 20 mg, 0.49 mmol). The flask was purged with nitrogen followed by addition of iodomethane (41 μL, 0.65 mmol). The resulting mixture was stirred under nitrogen until HPLC indicated complete consumption of **57**. The reaction mixture was diluted with diethyl ether and washed with water. The organic phase was dried over Na_2SO_4 and concentrated. ^1H NMR (400 MHz, $CDCl_3$) indicated a 1:1 mixture of **59** and **55**. A pure sample of **59** (41 mg, 31%) was obtained by RP HPLC. ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, 1 H, J = 7.7 Hz), 7.29-7.22 (m, 2 H), 5.74 (s, 1 H), 3.59 (m, 7 H), 3.25 (m, 4 H), 1.48 (s, 9 H). ES (+) MS m/e = 411 (M+1).

b) Title compound **60** was prepared according to the procedure of Example 16g,i,j except for using **59** as a reagent instead of **24**. ES (+) MS m/e = 549 (M+1).

Example 29

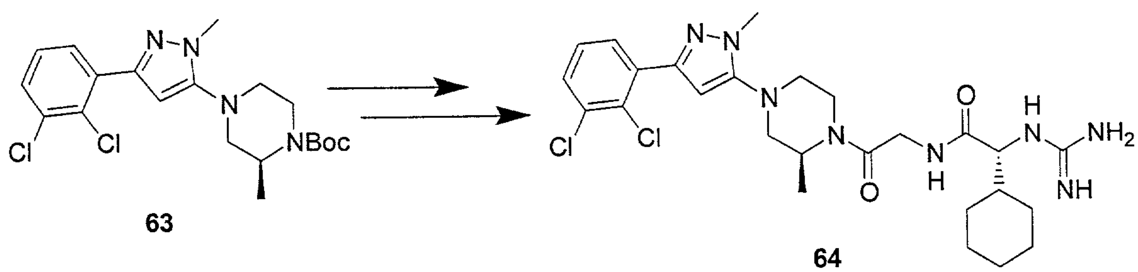


61 62

a) Compound **61** was prepared according to the procedure of Example 26c-e except for using 3-methyl-piperazine-1-carboxylic acid *tert*-butyl ester (prepared using literature procedures: Giardina et al. *J. Med. Chem.* **1993**, *36*, 690-698) as a reagent instead of piperazine-1-carboxylic acid *tert*-butyl ester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, 1 H, J = 7.8 Hz), 7.40 (d, 1 H, J = 7.9 Hz), 7.19 (app t, 1 H, J = 7.8 Hz), 6.40 (s, 1 H), 3.88 (m, 2 H), 3.79 (s, 3 H), 3.21 (app t, 1 H, J = 10.5 Hz), 3.00-2.82 (m, 3 H), 2.78 (app t, 1 H, J = 9.9 Hz), 1.47 (s, 3 H), 0.93 (d, 3 H, J = 5.6 Hz); ES (+) MS m/e = 425 (M+1).

b) Title compound **62** was prepared according to the procedure of Example 16g,i,j except for using **61** as a reagent instead of **24**. ES (+) MS m/e = 563 (M+1).

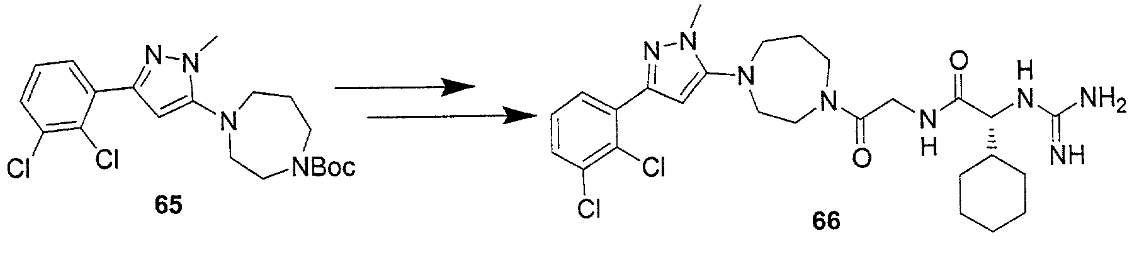
Example 30



a) Compound **63** was prepared according to the procedure of Example 26c-e except for using 2*S*-methylpiperazine-1-carboxylic acid *tert*-butyl ester as a reagent instead of piperazine-1-carboxylic acid *tert*-butyl ester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, 1 H, J = 7.8 Hz), 7.40 (d, 1 H, J = 7.9 Hz), 7.19 (app t, 1 H, J = 7.9 Hz), 6.29 (s, 1 H), 4.36 (br s, 1 H), 3.96 (d, 1 H, J = 12.9 Hz), 3.80 (s, 3 H), 3.25 (app t, 1 H, J = 12.6 Hz), 3.08 (d, 1 H, J = 11.0 Hz), 2.90 (app q, 2 H, J = 11.5 Hz), 2.74 (app t, 1 H, J = 11.8 Hz), 1.48 (s, 9 H), 1.18 (d, 3 H, J = 6.6 Hz); ES (+) MS m/e = 425 (M+1).

b) Title compound **64** was prepared according to the procedure of Example 16g,i,j except for using **63** as a reagent instead of **24**. ES (+) MS m/e = 563 (M+1).

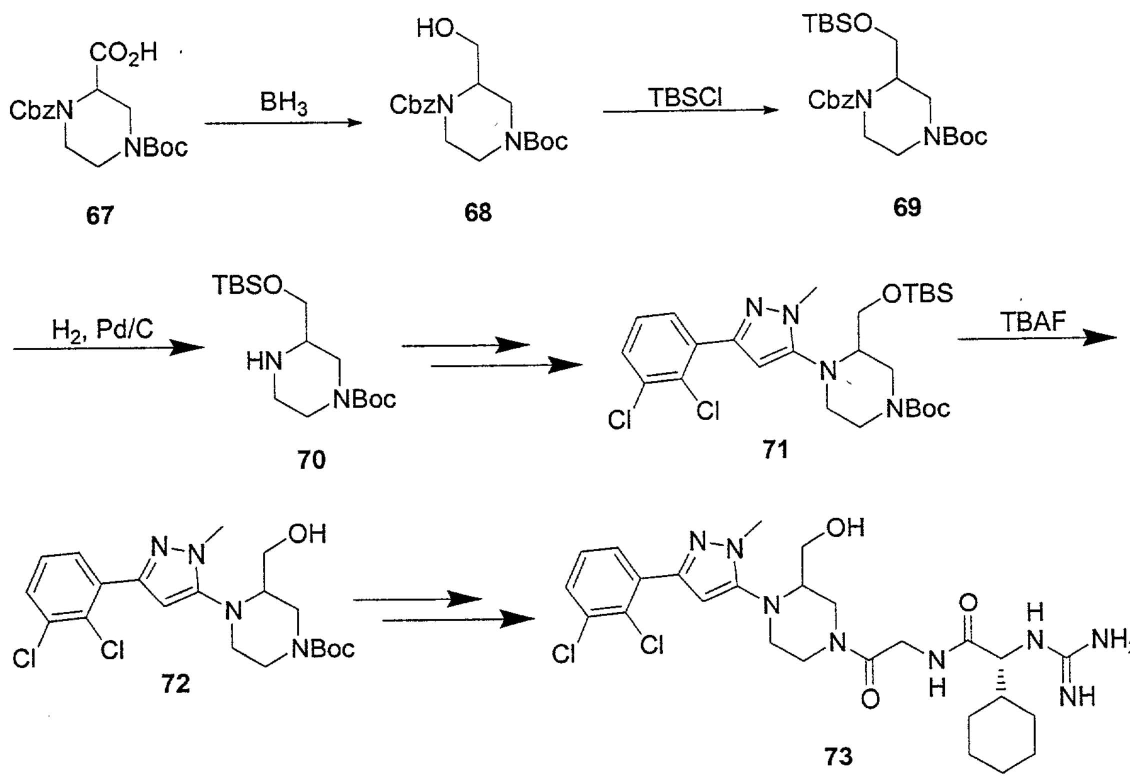
Example 31



a) Compound **65** was prepared according to the procedure of Example 26c-e except for using *tert*-butyl 1-homopiperazinecarboxylate as a reagent instead of piperazine-1-carboxylic acid *tert*-butyl ester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, 1 H, $J = 7.6$ Hz), 7.41 (d, 1 H, $J = 7.9$ Hz), 7.21 (app t, 1 H, $J = 7.8$ Hz), 6.31 (s, 1 H), 3.78 (s, 3 H), 3.63-3.53 (br m, 4 H), 3.17 (m, 4 H), 1.94 (m, 2 H), 1.48 (s, 9 H); TLC (SiO_2; 35% ethyl acetate in hexane): $R_f = 0.24$. ES (+) MS m/e = 425 (M+1).

b) Title compound **66** was prepared according to the procedure of Example 16g,i,j except for using **65** as a reagent instead of **24.** ES (+) MS m/e = 563 (M+1).

Example 32



a) To a solution of piperazine-1,2,4-tricarboxylic acid 1-benzyl ester 4-*tert*-butyl ester **(67)** (prepared by the method of Dorsey et al. *J. Med. Chem.* **1994**, *37*, 3443-3451) (8.6 g, 23.6 mmol) in dry THF (90 mL) at room temperature was added borane•THF (1.0M in THF, 70 mL, 70 mmol). The resulting mixture was stirred at 70°C for 1 hour and then cooled to room temperature. The reaction was quenched by dropwise addition of isopropanol and partitioned between saturated sodium bicarbonate (100 mL) and diethyl ether (100 mL). The aqueous layer was washed with diethyl ether (2 × 100 mL);

the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford **68** (8.2 g, 99%). ES (+) MS m/e = 373 (M+23).

b) To a solution of **68** (3.15 g, 9.0 mmol) and imidazole (0.67 g, 9.9 mmol) in dry DMF (18 mL) at room temperature was added *tert*-butyldimethylsilyl chloride (1.49 g, 9.9 mmol). The resulting mixture was stirred for 18 hours and then partitioned between water (100 mL) and ethyl acetate (100 mL). The aqueous layer was washed with ethyl acetate (2 X 50 mL); the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2: 10 to 20% ethyl acetate in hexanes) to afford **69** (4.1 g, 98%). ES (+) MS m/e = 487 (M+23).

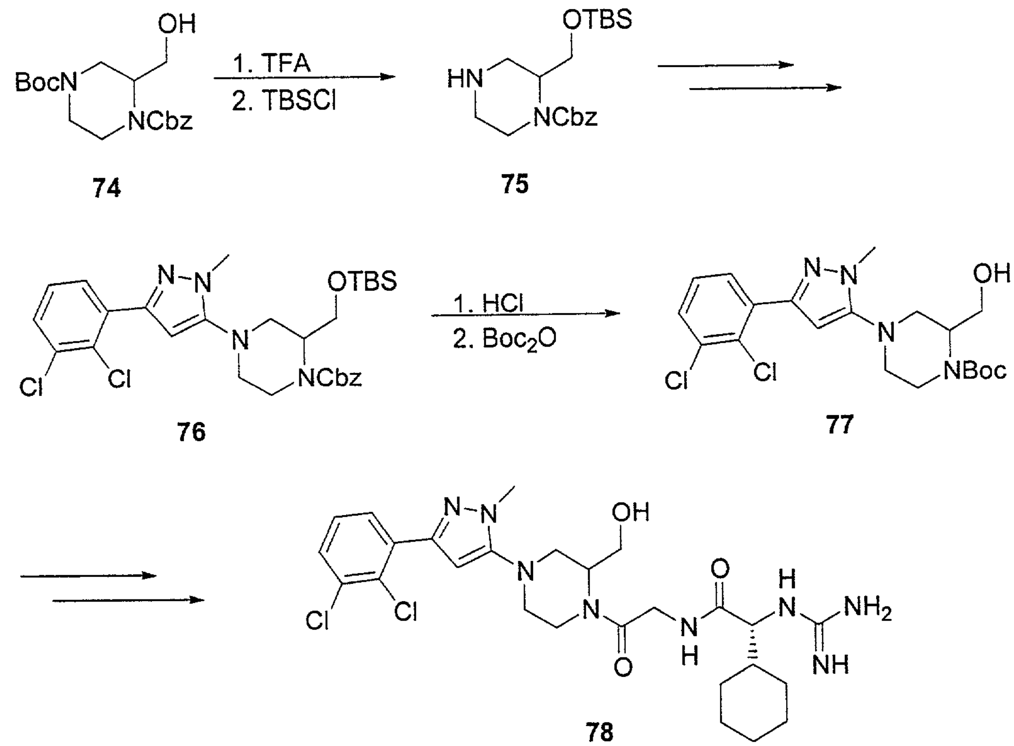
c) A solution of **69** (4.1g, 8.8 mmol) and 10% palladium on carbon (0.47 g, 0.44 mmol) in ethanol (30 mL) under an atmosphere of H_2 was shaken on a Parr hydrogenator (40 psi) for 1h. After filtration, the filtrate was concentrated under reduced pressure to afford **70** (2.73 g, 93%). ES (+) MS m/e = 331 (M+1).

d) Compound **71** was prepared according to the procedure of Example 26c-e except for using **70** as a reagent instead of piperazine-1-carboxylic acid *tert*-butyl ester. ES (+) MS m/e = 555 (M+1).

e) To a solution of **71** (0.268 g, 0.48 mmol) in THF (2.5 mL) at 0°C was added tetrabutylammonium fluoride (1.0M in THF, 0.96 mL, 0.96 mmol) dropwise. The resulting mixture was stirred for 30 minutes and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2: 50 to 100% ethyl acetate in hexane) to afford **72** (0.14 g). ES (+) MS m/e = 441 (M+1).

f) Title compound **73** was prepared according to the procedure of Example 16g,i,j except for using **72** as a reagent instead of **24.** ES (+) MS m/e = 579 (M+1).

Example 33



a) To a solution of **74** (4.26 g, 12.2 mmol) in dichloromethane (30 mL) was slowly added trifluoroacetic acid (8 mL). After stirring at ambient temperature for 45 min the reaction was diluted with 1,2-dichloroethane. The solvent was evaporated and the residue was dissolved in dichloromethane. To the resulting solution was added *tert*-butyldimethylsilyl chloride (5.50 g, 36.6 mmol), DMAP (300 mg, 2.44 mmol), and triethylamine (7.00 mL, 48.8 mmol). The mixture was stirred at room temperature overnight and then poured onto silica gel and purified by flash column chromatography (SiO_2: 0 to 6% methanol in dichloromethane) to yield **75** (3.40 g, 76%). TLC (SiO_2: 5% methanol in dichloromethane): R_f = 0.27; ES (+) MS m/e = 365 (M+1).

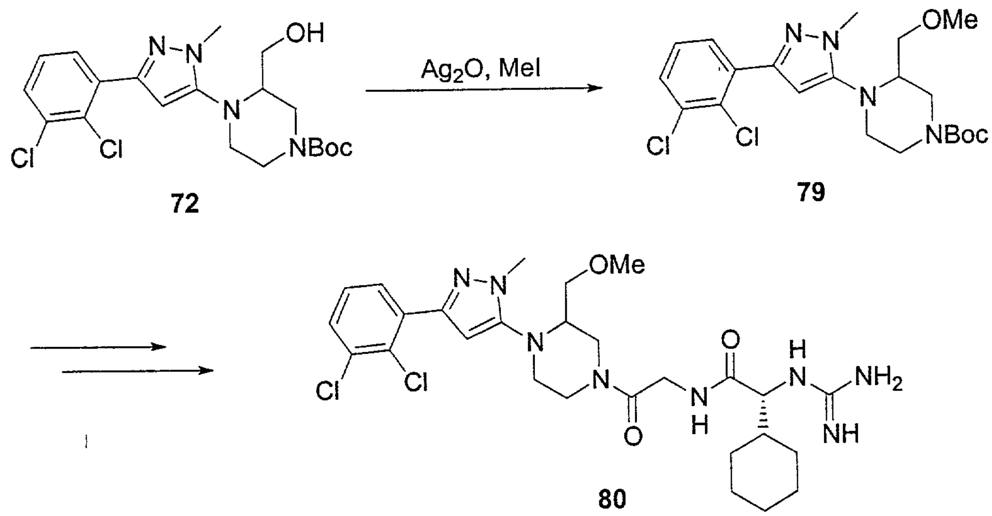
b) Compound **76** was prepared according to the procedure of Example 26c-e except for using **75** as a reagent instead of piperazine-1-carboxylic acid *tert*-butyl ester. TLC (SiO_2: 20% ethyl acetate in hexane): R_f = 0.24; ES (+) MS m/e = 589 (M+1).

c) A mixture of **76** (794 mg, 1.35 mmol) in aqueous HCl (6N, 10 mL) was heated at reflux until HPLC indicated complete deprotection of the starting material. The solvent was evaporated and the residue was dissolved in methanol (3.5 mL) and treated with triethylamine (1.5 mL) and di-*tert*-butyl dicarbonate (297 mg, 1.35 mmol) at room temperature. After 1h, the solvent was removed *in vacuo* and

the residue was purified by flash column chromatography (SiO_2: 20 to 90% ethyl acetate in hexane) to yield 77 (540 mg, 91%) as a white powder. TLC (SiO_2: 50% ethyl acetate in hexane): $R_f = 0.08$; ES (+) MS m/e = 441 (M+1).

Title compound 78 was prepared according to the procedure of Example 16g,i,j except for using 77 as a reagent instead of 24. ES (+) MS m/e = 579 (M+1).

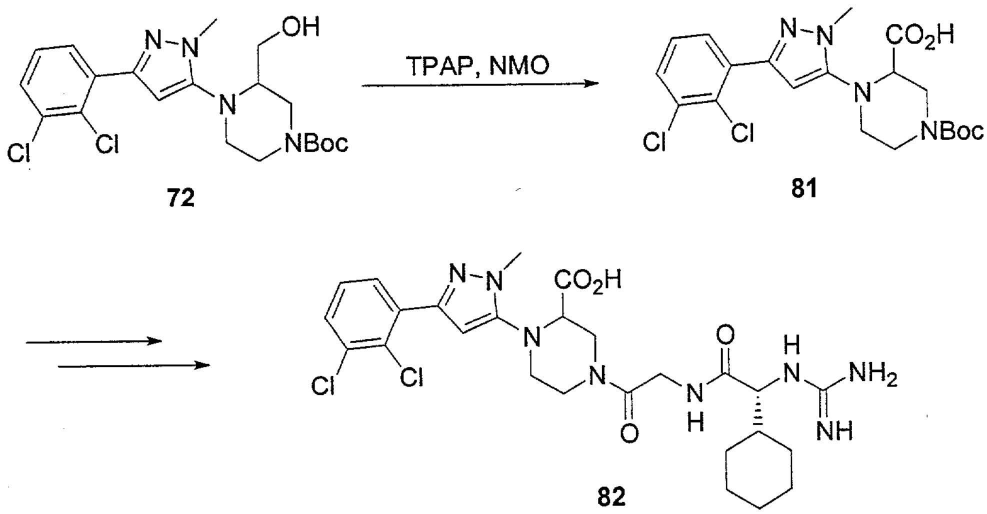
Example 34



a) To a solution of **72** (100 mg, 0.23 mmol) and silver (I) oxide (157 mg, 0.68 mmol) in acetonitrile (1.1 mL) was added iodomethane (0.28 mL, 4.6 mmol). The resulting mixture was heated at 40°C for 18 h and then cooled to room temperature. The heterogeneous solution was filtered and the filtrate concentrated under reduced pressure to afford crude **79** (0.1 g, 97%). ES (+) MS m/e = 455 (M+1).

b) Title compound **80** was prepared according to the procedure of Example 16g,i,j except for using 79 as a reagent instead of **24**. ES (+) MS m/e = 593 (M+1).

Example 35

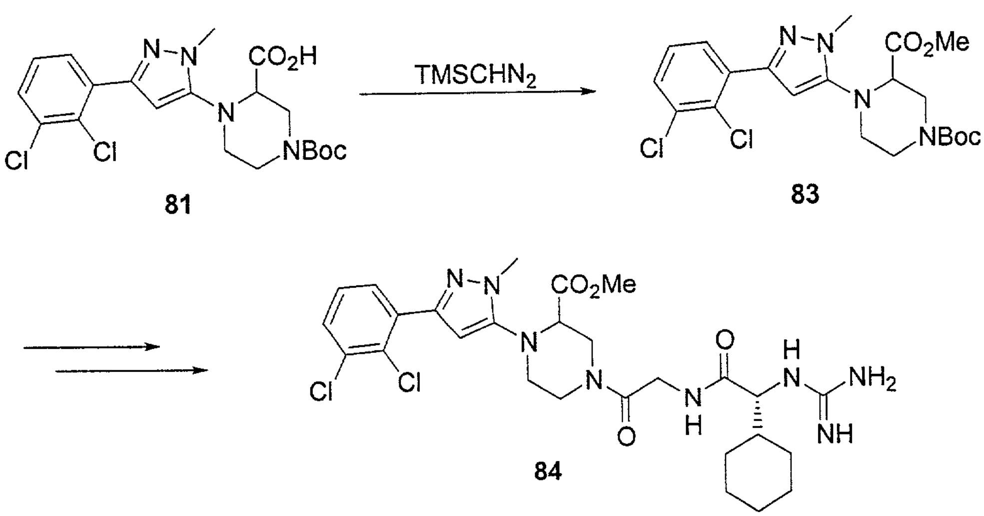


72 81 82

a) To a solution of **72** (110 mg, 0.25 mmol) and *N*-methylmorpholine-*N*-oxide (87 mg, 0.75 mmol) in wet acetonitrile (1.25 mL) was added tetrapropylammonium perruthenate (26 mg, 0.075 mmol). The resulting mixture was stirred at ambient temperature until LC/MS indicated complete conversion to product. The mixture was concentrated and purified by flash column chromatography (SiO_2: 0 to 10% methanol in dichloromethane) to yield **81** (60 mg, 53%). ES (+) MS m/e = 455 (M+1).

b) Title compound **82** was prepared according to the procedure of Example 16g,i,j except for using **81** as a reagent instead of **24**. ES (+) MS m/e = 593 (M+1).

Example 36

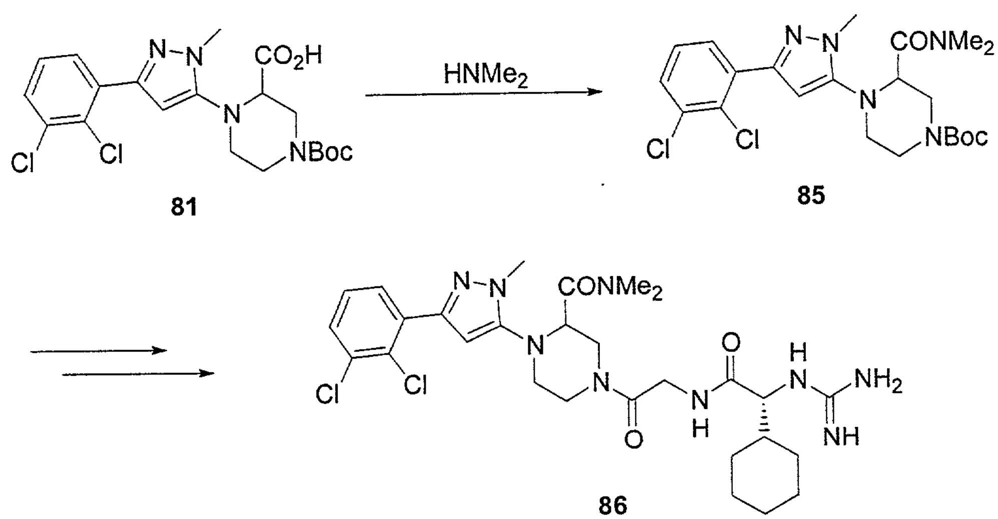


81 83 84

a) To a solution of **81** (45 mg, 0.09 mmol) in benzene / methanol (3:1, 5 mL) at room temperature was added trimethylsilyldiazomethane (2.0M in hexanes, 65 µL, 0.13 mmol). The resulting mixture was stirred for 15 minutes and concentrated under reduced pressure to afford crude **83** (46 mg, 100%). ES (+) MS m/e = 469 (M+1).

b) Title compound **84** was prepared according to the procedure of Example 16g,i,j except for using **83** as a reagent instead of **24**. ES (+) MS m/e = 607 (M+1).

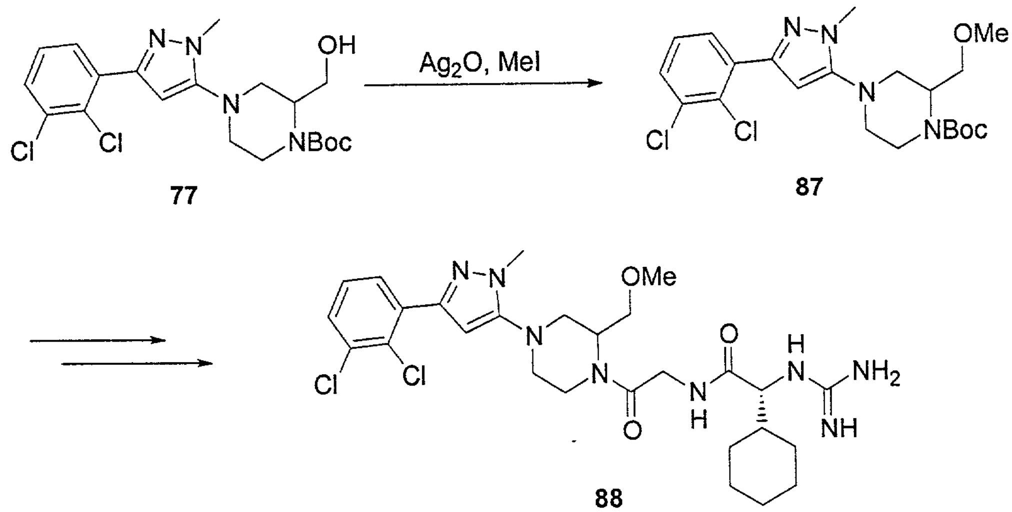
Example 37



a) To a solution of **81** (60 mg, 0.13 mmol) in dichloromethane (0.7 mL) at room temperature was added (chloromethylene)dimethyl ammonium chloride (36 mg, 0.28 mmol). The resulting mixture was stirred for 1 hour, followed by addition of dimethylamine (2.0M in THF, 0.13 mL, 0.26 mmol). The reaction stirred for 30 minutes and then partitioned between water (5 mL) and ethyl acetate (5 mL). The aqueous layer was washed with ethyl acetate (5 mL); the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford crude **85** (60 mg, 95%). ES (+) MS m/e = 482 (M+1).

b) Title compound **86** was prepared according to the procedure of Example 16g,i,j except for using **85** as a reagent instead of **24**. ES (+) MS m/e = 620 (M+1).

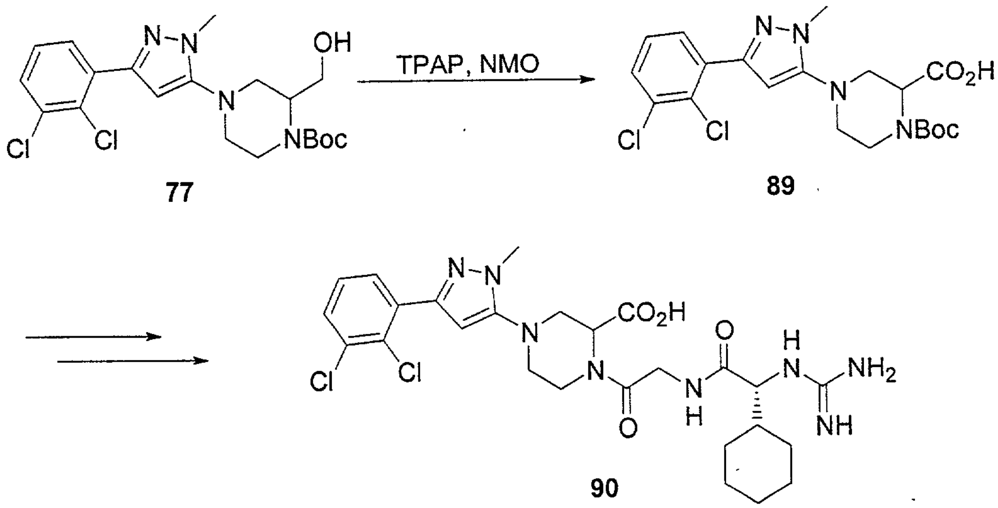
Example 38



a) A mixture of **77** (70.0 mg, 0.16 mmol), Ag_2O (110 mg, 0.48 mmol), and iodomethane (60 μL, 0.95 mmol) in acetonitrile (0.5 mL) was refluxed under nitrogen for 2 h. After cooling to room temperature, the mixture was filtered through a plug of Celite and concentrated to yield **87** (79 mg, over theory) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (dd, 1 H, J_1 = 7.8 Hz, J_2 = 1.5 Hz), 7.40 (dd, 1 H, J_1 = 7.9 Hz, J_2 = 1.5 Hz), 7.20 (app t, 1 H, J = 7.9 Hz), 6.29 (s, 1 H), 4.36 (br s, 1 H), 4.02 (m, 1 H), 3.88 (app t, 1 H, J = 9.3 Hz), 3.78 (s, 3 H), 3.46 (m, 1 H), 3.39 (s, 3 H), 3.34 (m, 1 H), 3.10 (m, 1 H), 3.07 (m, 1 H), 2.82-2.72 (m, 2 H), 1.48 (s, 9 H); ES (+) MS m/e = 455 (M+1).

b) Title compound **88** was prepared according to the procedure of Example 16g,i,j except for using **87** as a reagent instead of **24**. ES (+) MS m/e = 593 (M+1).

Example 39



77

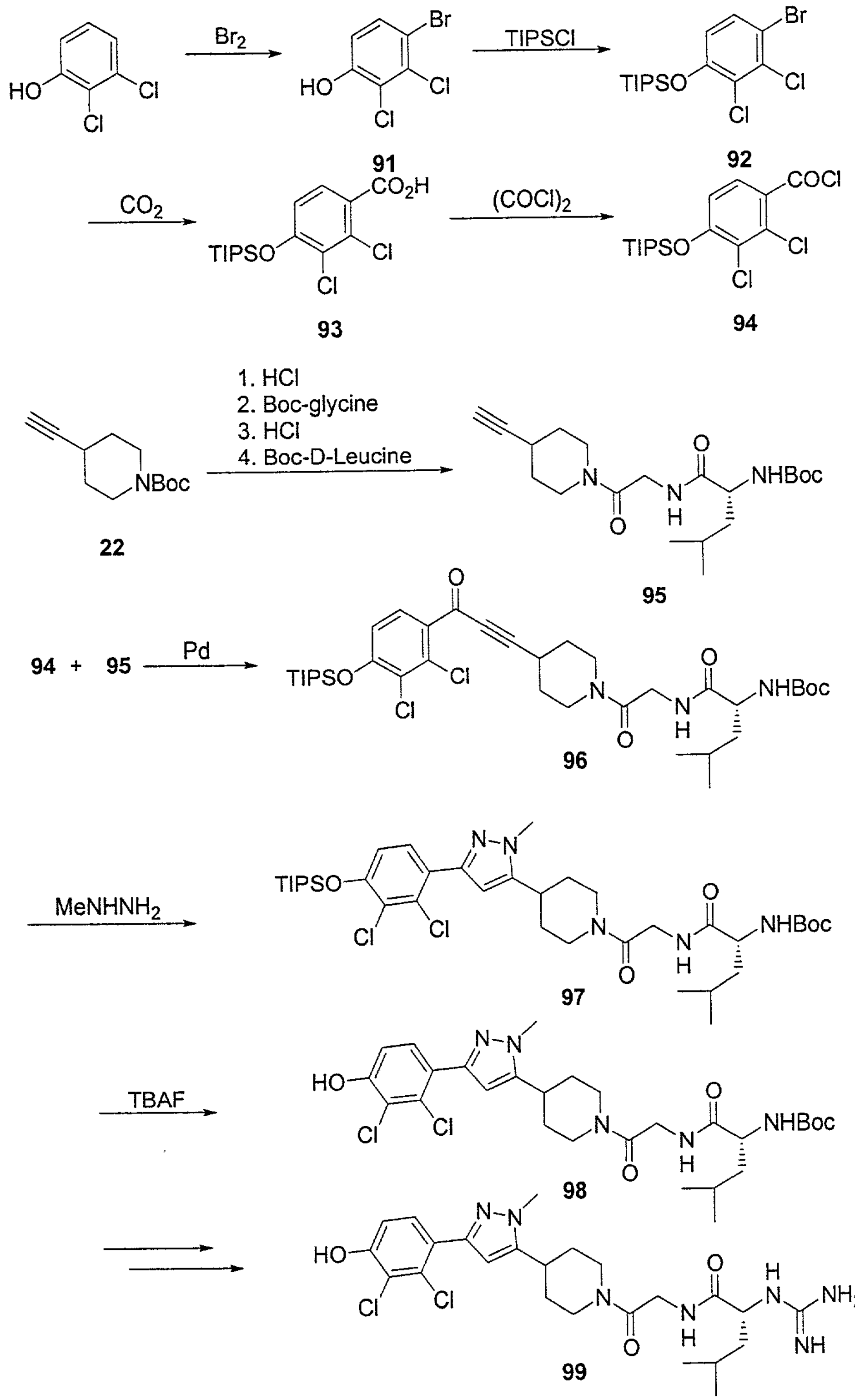
89

90

a) Compound **89** was prepared according to the procedure of Example 35a except for using **77** (100 mg, 0.23 mmol) as a reagent instead of **72**. TLC (SiO_2: 10% methanol in dichloromethane): $R_f = 0.28$; ES (+) MS m/e = 455 (M+1).

b) Title compound **90** was prepared according to the procedure of Example 16g,i,j except for using **89** as a reagent instead of **24**. ES (+) MS m/e = 593 (M+1).

Example 40


Br2
TIPSCl
91
92
CO2
(COCl)2
93
94
1. HCl
2. Boc-glycine
3. HCl
4. Boc-D-Leucine
22
95
94 + 95
Pd
96
MeNHNH2
97
TBAF
98
99

a) To a solution of 2,3-dichlorophenol (10.0 g, 61.3 mmol) in dichloromethane (50 mL) was added bromine (4.11 mL, 79.8 mmol) dropwise. After 2h, HPLC indicated complete consumption of the starting material. The reaction mixture was slowly poured into a 10% aqueous sodium thiosulfate solution. The phases were separated and the aqueous layer was extracted with dichloromethane (3×). The combined organic layer was dried over Na_2SO_4 and concentrated. Purification of the crude residue by flash column chromatography (SiO_2: 0 to 20% ethyl acetate in hexane) yielded 91 (7.86 g, 53%) as a white solid. Spectroscopic data is identical to that of commercially available 4-bromo-2,3-dichloro-phenol from ChemService Inc. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, 1 H, J = 8.9 Hz), 6.86 (d, 1 H, J = 8.9 Hz), 5.72 (s, 1 H); TLC (SiO_2: 20% ethyl acetate in hexane): R_f = 0.29.

b) To a solution of **91** (11.7 g, 48.4 mmol) in dry THF (100 mL) was added triethylamine (7.4 mL, 53.2 mmol) followed by triisopropylsilyl chloride (11.4 mL, 53.2 mmol). The reaction was stirred for 1 h at room temperature. Water (250 mL) and ethyl acetate (250 mL) were added and the layers separated. The aqueous layer was washed with additional ethyl acetate (200 mL). The organic layers were combined, dried over $MgSO_4$, filtered through a short plug of silica gel, concentrated under reduced pressure, and dried under high vacuum at 50°C for 48h to yield **92** (19.0 g, 99%) of a viscous oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.36 (d, 1 H, J = 8.9 Hz), 6.72 (d, 1 H, J = 8.9 Hz), 1.32 – 1.27 (m, 3 H), 1.10 (d, 18 H, J = 7.7 Hz).

c) To a –78°C solution of **92** (15.4 g, 38.7 mmol) in anhydrous THF (385 mL) under nitrogen was added *n*-butyl lithium (1.57M in hexane, 24.6 mL, 38.7 mmol) dropwise. After 10 min at –78°C, carbon dioxide was bubbled through the solution for approximately 10 minutes. The mixture was allowed to reach room temperature and was quenched by careful addition of water (310 mL) followed by aqueous HCl (1.0N, 38.7 mL, 38.7 mmol). The mixture was extracted with diethyl ether (3×) and the combined organic layer was dried over $MgSO_4$ and concentrated. Purification of the crude residue by flash column chromatography (SiO_2: 0 to 70% ethyl acetate in hexane) yielded **93** (6.65 g, 47%) as a white solid. TLC (SiO_2: 30% ethyl acetate in hexane): R_f = 0.39; ES (+) MS m/e = 363 (M+1).

d) To a solution of **93** (6.45 g, 17.8 mmol) in anhydrous dichloromethane (120 mL) under nitrogen was added DMF (1.38 mL, 17.8 mmol) followed by dropwise addition of oxalyl chloride (2.01 mL, 23.1 mmol). After 10 min HPLC indicated complete consumption of the starting material and 1,2-dichloroethane was added. The solvent was removed *in vacuo* and the excess oxalyl chloride was

removed by co-evaporation with 1,2-dichloroethane. The residue was dried under high vacuum to provide **94** which was used without further purification.

e) A solution of **22** (1.5 g, 7.0 mmol) in HCl/dioxane (4.0N, 10 mL) was stirred at room temperature for 30 minutes. The solvent was removed under reduced pressure to afford the desired amine (1.0 g, 100%) as the hydrochloride salt.

To *N*-Boc-glycine (1.5 g, 8.4 mmol) in dichloromethane (30 mL) was added EDC (1.6 g, 8.4 mmol), HOBt monohydrate (1.3 g, 8.4 mmol) and triethylamine (2.4 mL, 16.9 mmol). The piperidine amine (1.0 g, 7.0 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was partitioned with water (20 mL) and separated. The aqueous layer was extracted with dichloromethane (2 × 20 mL), and the combined organic layer was washed with 1M HCl (30 mL), saturated $NaHCO_3$ (30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to yield the desired amide (1.8 g, 96%).

To the amide (1.8 g, 6.8 mmol) was added HCl/dioxane (4.0N, 10 mL) and the reaction was stirred at room temperature for 30 minutes. The solvent was removed under reduced pressure to give the desired amine (1.4 g, 100%) as the hydrochloride salt.

To *N*-Boc-D-leucine (1.9 g, 8.1 mmol) in dichloromethane (30 mL) was added EDC (1.6 g, 8.1 mmol), HOBt monohydrate (1.2 g, 8.1 mmol) and triethylamine (2.2 mL, 16.3 mmol). To the activated acid solution was added amine (1.4 g, 6.8 mmol) and the reaction was stirred at room temperature for 4h. The reaction mixture was partitioned with water (20 mL) and separated. The aqueous layer was extracted with dichloromethane (2 × 20 mL), and the combined organic layer was washed with 1M HCl (30 mL), saturated $NaHCO_3$ (30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to provide the crude amide (2.2 g); purification by flash chromatography (SiO_2: 25% ethyl acetate in hexanes) provided 95 (1.1 g).

f) A round bottom flask containing **94** (~17.8 mmol), CuI (0.17 g, 0.89 mmol), $PdCl_2(PPh_3)_2$ (0.63 mg, 0.89 mmol), and **95** (6.75 g, 17.8 mmol) was flushed with nitrogen for several minutes and then charged with a degassed solution of triethylamine (4.97 mL, 35.6 mmol) in toluene (120 mL). The reaction was monitored by HPLC and the solvent removed was removed when 94 had been completely consumed. The crude product **96** obtained was used without further purification.

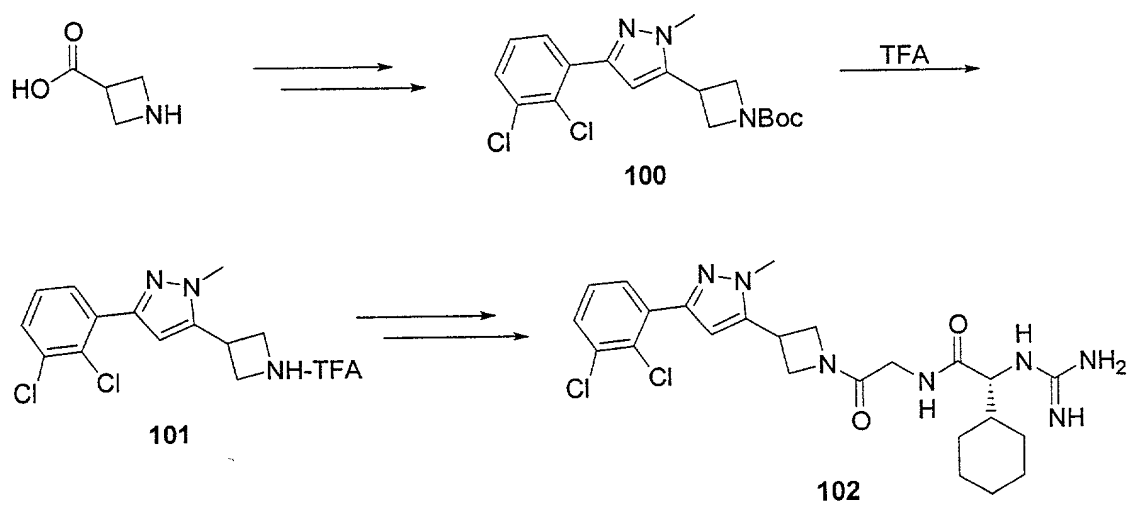
g) A solution of **96** and methylhydrazine (9.47 mL, 178 mmol) in ethanol (120 mL) was stirred at ambient temperature for 1.5 h. The reaction mixture was concentrated and then redissolved in dichloromethane. The organic phase was washed with water and the resulting aqueous layer was

extracted with dichloromethane (2×). The combined organic layer was dried over Na_2SO_4 and concentrated. Purification of the crude residue by flash column chromatography (SiO_2: 50 to 100% ethyl acetate in hexane) provided **97** (7.0 g, 52% for three steps from **93**) as a white foam. TLC (SiO_2: ethyl acetate): $R_f = 0.19$; ES (+) MS m/e = 752 (M+1).

h) To a 0°C solution of **97** (7.0 g, 9.3 mmol) in anhydrous THF (50 mL) was added tetrabutylammonium fluoride (1.0M in THF, 14 mL, 14 mmol) dropwise. The reaction mixture was stirred for 30 min and then partitioned between water (200 mL) and ethyl acetate (200 mL). The aqueous layer was washed with ethyl acetate (3 × 200 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the crude product by flash column chromatography (SiO_2: 10% methanol in ethyl acetate) yielded **98** (5.0 g, 90%) as a solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (d, 1 H, J = 8.5 Hz), 7.14 (d, 1 H, J = 8.8 Hz), 6.40 (s, 1 H), 4.61 (d, 1 H, J = 12.8 Hz), 4.16-4.05 (m, 4 H), 3.88 (s, 3 H), 3.25 (m, 1 H), 3.06 (m, 1 H), 2.83 (m, 1 H), 2.01 (m, 2 H), 1.71-1.53 (m, 6 H), 1.44 (s, 9 H), 2.83 (m, 6 H); TLC (SiO_2: 5% methanol in dichloromethane): $R_f = 0.10$; ES (+) MS m/e = 596 (M+1).

i) Title compound **99** was prepared according to the procedure of Example 16j except for using **98** as a reagent instead of **27**. ES (+) MS m/e = 538 (M+1).

Example 41

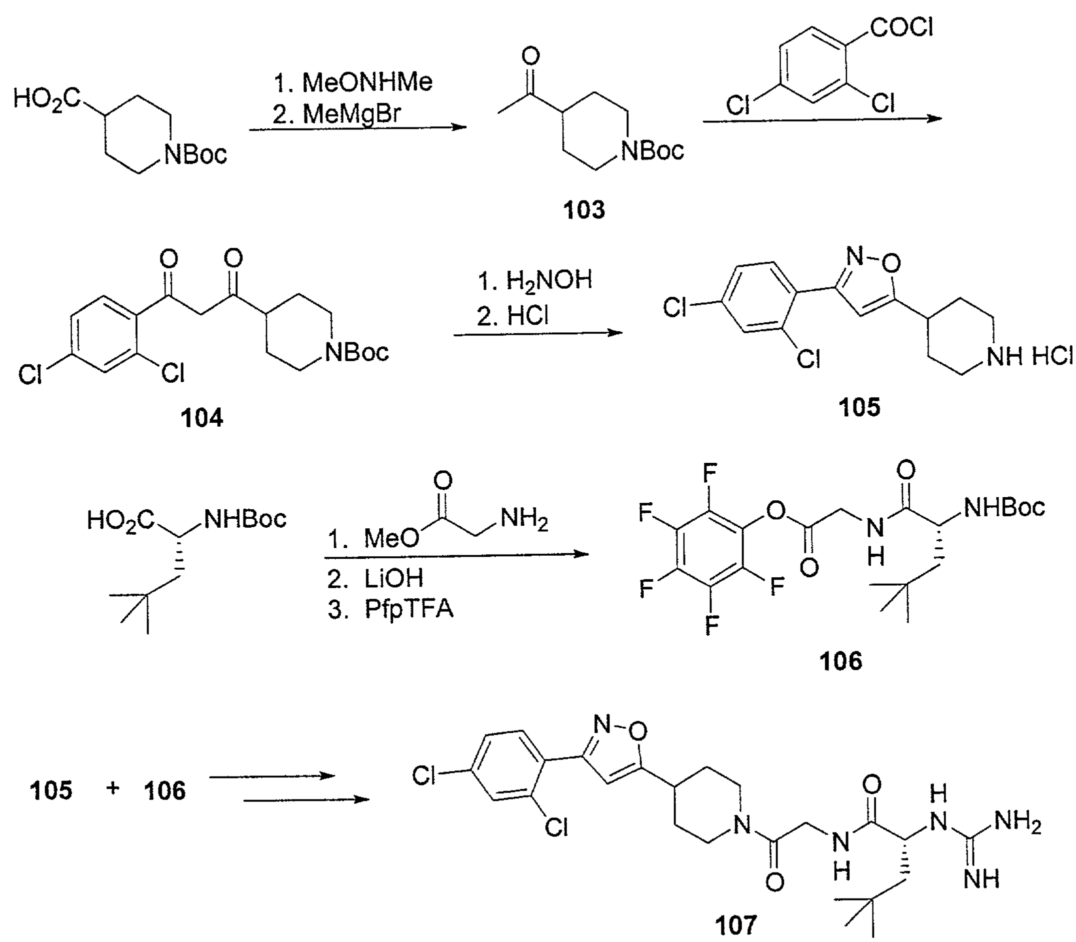


a) Compound **100** was prepared according to the procedure of Example 16a-f except for using azetidine-3-carboxylic acid as a reagent instead of piperidine-4-carboxylic acid.

b) To a solution of **100** (3.46 g, 9.1mmol) in dichloromethane (20 mL) was added trifluoroacetic acid (5 mL). The resulting mixture was stirred for 3 hours at room temperature and concentrated under reduced pressure to afford crude **101** (4.4 g, 98%). ES (+) MS m/e = 282 (M+1).

c) Title compound **102** was prepared according to the procedure of Example 16i,j except for using **101** as a reagent instead of **25**. ES (+) MS m/e = 520 (M+1).

Example 42



a) To *N*-Boc-isonipecotic acid (40 g, 0.17 mol) in dichloromethane (800 mL) was added EDC (36.8 g, 0.192 mol), HOBt monohydrate (29.4 g, 0.192 mol), triethylamine (51.0 mL, 0.366 mol) and *N,O*-dimethylhydroxylamine hydrochloride (18.7 g, 0.192 mol). The reaction mixture was stirred overnight at room temperature and then partitioned between water and dichloromethane. The aqueous

layer was extracted with dichloromethane (3 × 200 mL); the combined organic layer was washed with 1M HCl (300 mL), saturated $NaHCO_3$ (300 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the desired amide (41.4 g, 87%).

b) To the amide (24.0 g, 88.2 mmol) in anhydrous THF (320 mL) at –78°C under nitrogen was added *via* syringe over 10 minutes methyl magnesium bromide (1M in THF, 106 mL, 106 mmol). The reaction mixture was warmed to 0°C, stirred for 2 h, and then quenched with 1M HCl (150 mL). The mixture was extracted with ethyl acetate (3 × 200 mL) and the combined organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to yield ketone 103 (19.4 g, 97%) as a yellow oil. ES (+) MS m/e = 154 (M-55).

c) To a solution of **103** (500 mg, 2.2 mmol) in anhydrous THF (9 mL) at –78°C under nitrogen was added dropwise *via* syringe lithium diisopropylamide (2.0M in heptane/THF/ether, 1.2 mL, 2.4 mmol). The mixture was stirred at –78°C for 30 minutes, and then 2,4-dichlorobenzoyl chloride (339 μL, 2.4 mmol) in THF (1 mL) was added dropwise. The reaction mixture was warmed to –10°C, stirred for 2 h, and then quenched with water (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 20 mL); the combined organic layer was washed with 1M HCl (10 mL), saturated $NaHCO_3$ (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give 839 mg of crude material which was purified by flash chromatography (SiO_2: 25% ethyl acetate in hexanes followed by 50% MeOH in ethyl acetate) to afford **104** (250 mg, 28%). ES (+) MS m/e = 344 (M-55).

d) To a solution of **104** (125 mg, 0.31 mmol) in MeOH (2 mL) was added hydroxylamine (16M in H_2O, 63 μL, 1.0 mmol). The reaction mixture was refluxed for 4h and the solvent was removed under reduced pressure. The resulting residue was partitioned between ethyl acetate (10 mL) and water (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL); the combined organic layer was washed with 1M HCl (10 mL) and dried over Na_2SO_4. The solvent was removed under vacuum to afford the isoxazole (119 mg, 96%) as roughly a 1:1 mixture of regioisomers which was used without further purification.

The isoxazole was dissolved in HCl/dioxane (4.0N, 2 mL) and stirred at room temperature for 30 min. The solvent was removed under vacuum to yield **105** (101 mg, 100%). ES (+) MS m/e = 298 (M+1).

e) To a solution of *N*-Boc-D-*t*-butylalanine (5.0 g, 20.3 mmol) in dichloromethane (80 mL) was added EDC (4.7 g, 24.4 mmol), HOBt (3.3 g, 24.4 mmol), triethylamine (6.2 mL, 44.7 mmol), and then glycine methyl ester hydrochloride (3.1 g, 24.4 mmol). The reaction mixture was stirred overnight at

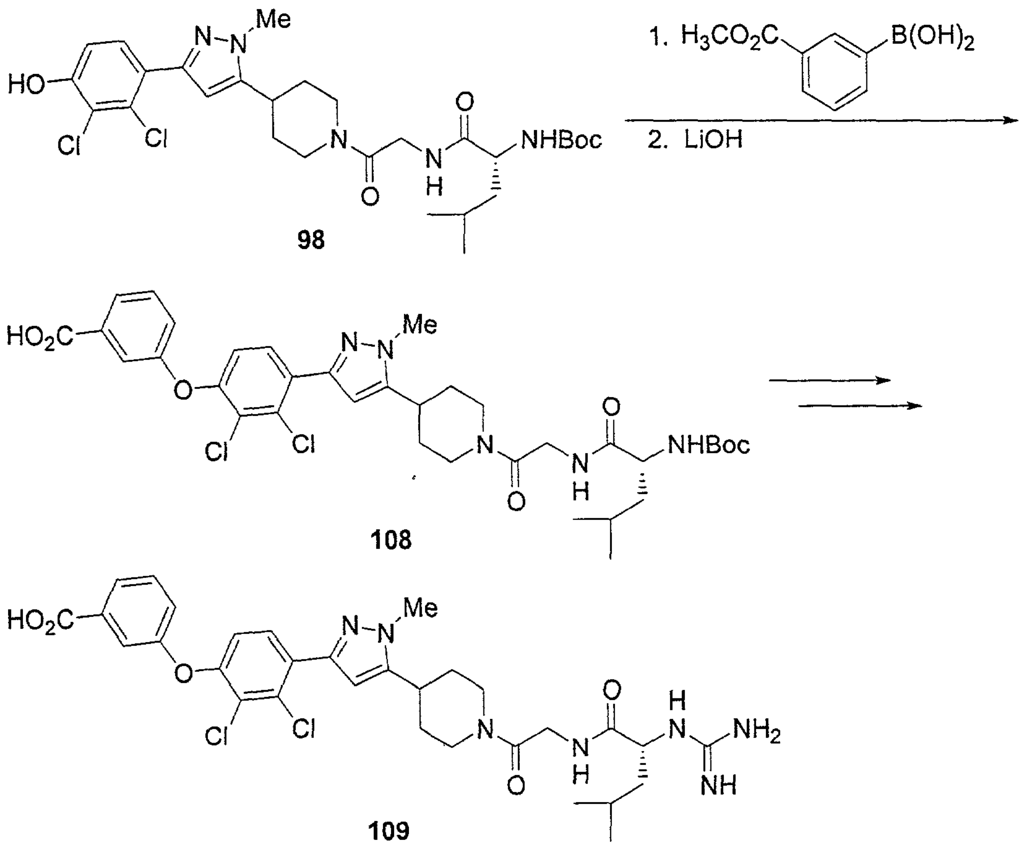
room temperature and then partitioned between water (50 mL) and dichloromethane (20 mL). The aqueous layer was extracted with dichloromethane (2 × 50 mL); the combined organic layer was washed with 1M HCl (50 mL), saturated $NaHCO_3$ (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the desired amide (6.1 g, 95%).

The amide was dissolved in THF/H_2O (3:1, 80 mL) and lithium hydroxide monohydrate (1.6 g, 38.3 mmol) was added. The reaction was stirred overnight at room temperature and then neutralized with 1M HCl (50 mL). The mixture was extracted with ethyl acetate (3 × 100 mL) and the combined organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure to yield the carboxylic acid (5.8 g, 100%).

To a solution of the acid in THF (80 mL) was added pyridine (1.7 mL, 21.1 mmol) and pentafluorophenyl trifluoroacetate (3.6 mL, 21.1 mmol). The reaction was incomplete after stirring at room temperature overnight. Additional pentafluorophenyl trifluoroacetate (3.6 mL, 21.1 mmol) was used to drive the reaction to completion (2 h). The solvent was removed under reduced pressure and the resulting residue was dissolved in ethyl acetate (100 mL) and washed with 1M HCl (60 mL) followed by saturated $NaHCO_3$ (60 mL). The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to afford 14.0 g of crude material. The crude product was purified by flash chromatography (SiO_2: 50% ethyl acetate in hexane) to provide **106** (11.4 g) which was used without further purification. ES (+) MS m/e = 413 (M-55).

f) Title compound **107** was prepared according to the procedure of Example 16g,i,j except for using **105** and **106** as reagents instead of **25** and **26**. ES (+) MS m/e = 524 (M+1).

Example 43

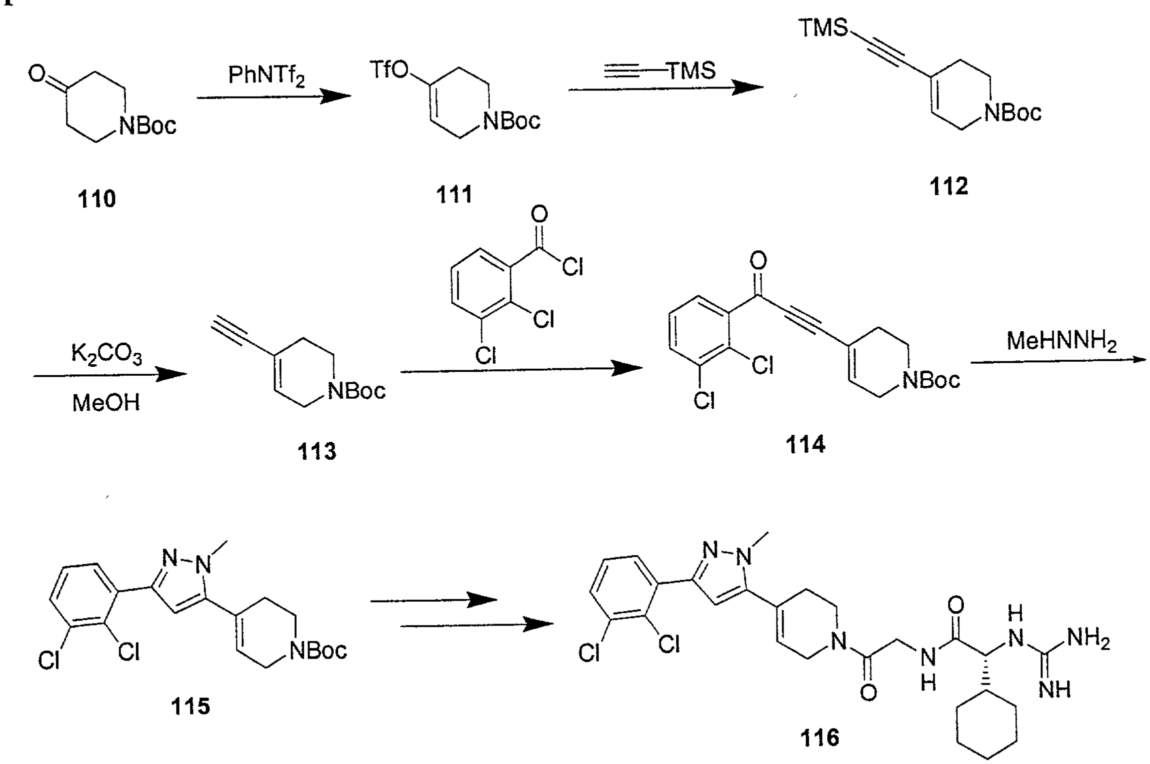


a) To phenol **98** (120 mg, 0.20 mmol) in dichloromethane (2 mL) was added (*m*-carbomethoxy)-phenylboronic acid (67 mg, 0.40 mmol), $Cu(OAc)_2$ (36 mg, 0.20 mmol), triethylamine (139 μL, 1.0 mmol) and molecular sieves (spatula tip). The reaction mixture was stirred at room temperature overnight and then filtered. The filtrate was diluted with dichloromethane (5 mL) and washed with water (5 mL). The aqueous layer was extracted with dichloromethane (3 × 5 mL); the combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford the biaryl ether (250 mg) which was used without further purification.

To a solution of the aryl ether in THF / H_2O (3:1, 3 mL) was added lithium hydroxide (23 mg, 1.0 mmol). The reaction mixture was stirred at room temperature overnight and then neutralized with 1M HCl (4 mL). The mixture was extracted with ethyl acetate (3 X 5 mL); the combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield the desired carboxylic acid **108**. ES (+) MS m/e = 716 (M+1).

b) Title compound **109** was prepared according to the procedure of Example 16j except for using **108** as a reagent instead of **27**. ES (+) MS m/e = 658 (M+1).

Example 44



a) To a solution of **110** (5.0 g, 25.0 mmol) in dry THF (50 mL) at -78°C was added lithium diisopropylamide (2.0M in heptane/THF/ethylbenzene, 19 mL, 38 mmol)) dropwise. The resulting mixture was stirred at -78°C for 10 minutes followed by addition of *N*-phenyltrifluoromethane-sulfonimide (10.0 g, 28.0 mmol). The reaction was warmed to room temperature, stirred for 18 hours, and then partitioned between water (100 mL) and ethyl acetate (100 mL). The aqueous layer was washed with ethyl acetate (2 X 100 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2: 15% ethyl acetate in hexanes) to afford **111** (6.32 g, 76%). ES (+) MS m/e = 276 (M+1).

b) To a solution of **111** (1.0 g, 3.0 mmol), copper (I) iodide (29 mg, 0.15 mmol), (trimethylsilyl)acetylene (0.36 g, 3.6 mmol) and triethylamine (0.42 mL, 3.0 mmol) in toluene (15 mL) at room temperature was added dichlorobis(triphenylphosphine)palladium(II) (0.106 g, 0.15 mmol). The resulting mixture was stirred for 2 hours and concentrated under reduced pressure. The residue was

purified by flash chromatography (SiO_2: 10% ethyl acetate in hexanes) to afford **112** (0.75 g, 89%). ES (+) MS m/e = 224 (M-56).

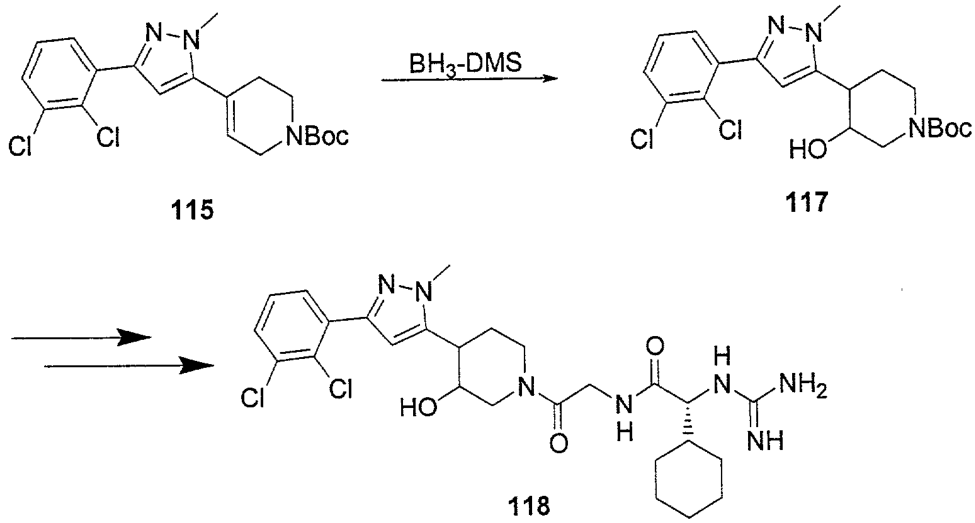
c) To a solution of **112** (0.75 g, 2.7 mmol) in methanol (15 mL) at room temperature was added potassium carbonate (0.75 g, 5.4 mmol). The resulting mixture was stirred for 1 hour and then partitioned between water (50 mL) and ethyl acetate (50 mL). The aqueous layer was washed with ethyl acetate (2 × 50 mL); the combined organic layer were dried over $MgSO_4$ and concentrated under reduced pressure to afford crude **113** (0.53 g, 94%). ES (+) MS m/e = 152 (M-56).

d) To a solution of **113** (0.52 g, 2.5 mmol), copper (I) iodide (0.024 g, 0.12 mmol), 2,3-dichlorobenzoyl chloride (0.68 g, 3.3 mmol) and triethylamine (0.45 mL, 3.3 mmol) in toluene (12 mL) at room temperature was added dichlorobis(triphenylphosphine)palladium(II) (88 mg, 0.12 mmol). The resulting mixture was stirred for 3 hours and then partitioned between saturated sodium bicarbonate (50 mL) and ethyl acetate (50 mL). The aqueous layer was washed with ethyl acetate (2 × 50 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2: 25% ethyl acetate in hexanes) to afford **114** (0.93 g, 98%). ES (+) MS m/e = 324 (M-56).

e) To a solution of **114** (0.93 g, 2.5 mmol) in ethanol (10 mL) at room temperature was added methylhydrazine (0.22 g, 4.9 mmol). The resulting mixture was stirred for 30 minutes and then concentrated under reduced pressure. The residue was purified by chromatography (SiO_2: 25% to 40% ethyl acetate in hexanes) to afford **115** (0.85 g, 85%) as a single regioisomer. ES (+) MS m/e = 408 (M+1).

f) Title compound **116** was prepared according to the procedure of Example 16g,i,j except for using **115** as a reagent instead of **24**. ES (+) MS m/e = 546 (M+1).

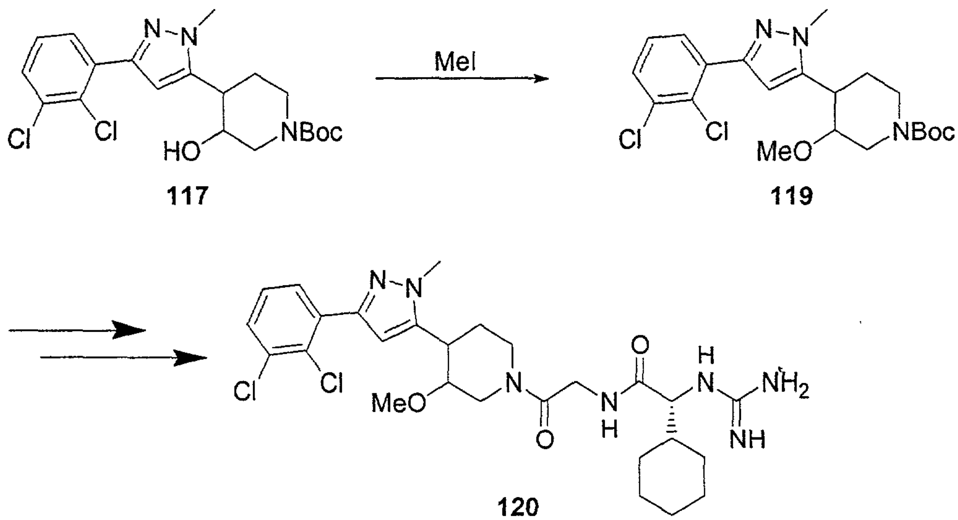
Example 45



a) To a solution containing borane•THF (1.0M in THF, 4.4 mL, 4.4 mmol) in THF (9 mL) at 0°C was added a solution of **115** (0.9 g, 2.2 mmol) in THF (2 mL). The resulting mixture was stirred for 4 h at room temperature and then treated with 3M NaOH/30% H_2O_2(aq) (1:1, 2 mL). After 15 min, the solution was partitioned between water (50 mL) and ethyl acetate (50 mL). The aqueous layer was washed with ethyl acetate (2 X 50 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by RP HPLC to afford **117** (0.26 g, 28%). ES (+) MS m/e = 426 (M+1).

b) Title compound **118** was prepared according to the procedure of Example 16g,i,j except for using **117** as a reagent instead of **24**. ES (+) MS m/e = 564 (M+1).

Example 46

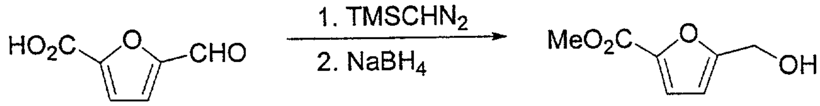


117 119 120

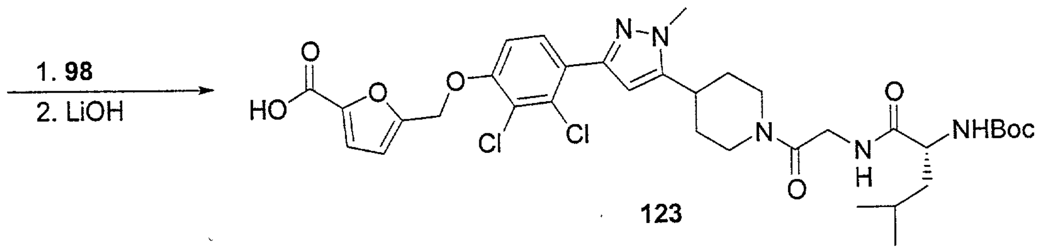
a) To a solution of **117** (50 mg, 0.11 mmol) in DMF (1 mL) at 0°C was added sodium hydride (60% wt in mineral oil, 7 mg, 0.18 mmol) followed by iodomethane (12 μL, 0.17 mmol). The reaction mixture was stirred for 1 hour at room temperature and then partitioned between water (5 mL) and ethyl acetate (5 mL). The aqueous layer was washed with ethyl acetate (2 X 5 mL); the combined organic layer was dried and concentrated under reduced pressure to afford crude **119** (30 mg, 59%). ES (+) MS m/e = 440 (M+1).

b) Title compound **120** was prepared according to the procedure of Example 16g,i,j except for using **119** as a reagent instead of **24**. ES (+) MS m/e = 578 (M+1).

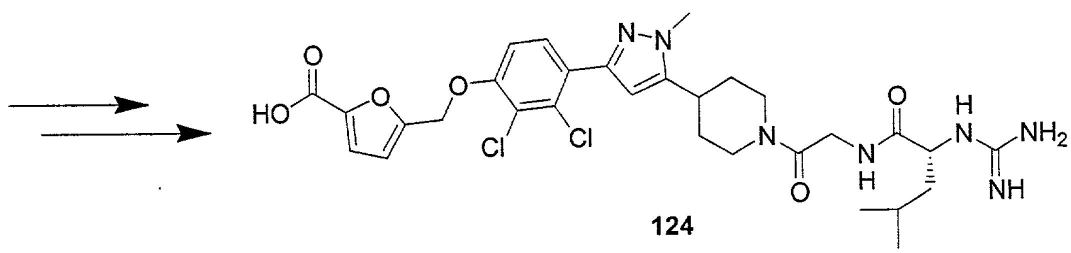
Example 47



121 122



123



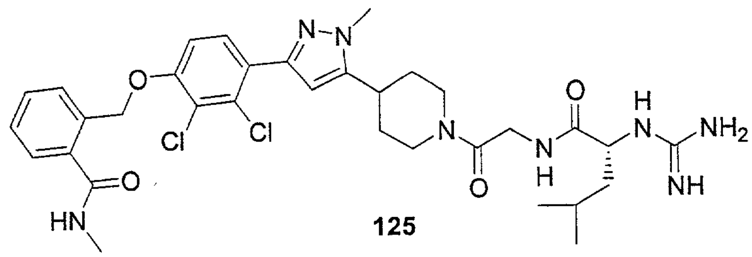
124

a) To a solution of **121** (0.15 g, 1.1 mmol) in benzene / methanol (5:1, 2 mL) at room temperature was added (trimethylsilyl)diazomethane (2.0M in hexanes, 0.6 mL, 1.2 mmol). The resulting solution was stirred at room temperature for 1 hour and concentrated under reduced pressure. The residue was dissolved in methanol (1 mL) and treated with sodium borohydride (80 mg, 2.1 mmol). After 2 h the reaction mixture was partitioned between water (10 mL) and ethyl acetate (10 mL). The aqueous layer was washed with ethyl acetate (2 X 10 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2: 10 to 50% ethyl acetate in hexanes) to afford **122** (37 mg, 22%). ES (+) MS m/e = 157 (M+1).

b) To a solution of **98** (70 mg, 0.12 mmol), **122** (37 mg, 0.23 mmol), and triphenylphosphine (61 mg, 0.23 mmol) in THF (1 mL) at room temperature was added diethyl azodicarboxylate (37 µL, 0.23 mmol). The resulting solution was stirred for 1 hour and then concentrated under reduced pressure. The residue was dissolved in THF (0.5 mL) and 1M LiOH (2 mL) and heated to 60°C for 18 hours. The reaction mixture was cooled, concentrated under reduced pressure, and purified by RP HPLC to afford **123** (42 mg, 49%). ES (+) MS m/e = 720 (M+1).

c) Title compound **124** was prepared according to the procedure of Example 16j except for using **123** as a reagent instead of **27**. ES (+) MS m/e = 662 (M+1).

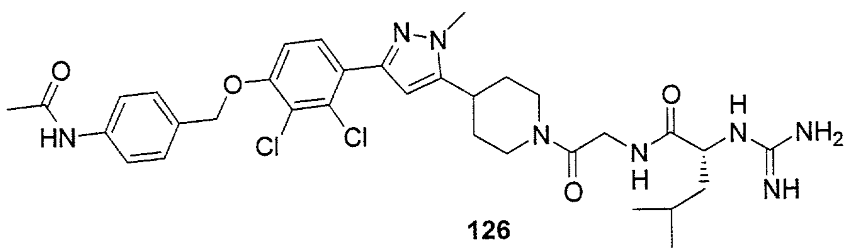
Example 48



125

Title compound **125** was prepared from **98** according to the procedure of Example 48b,c except for using 2-hydroxymethyl-*N*-methylbenzamide as a reagent instead of **122**. ES (+) MS m/e = 685 (M+1).

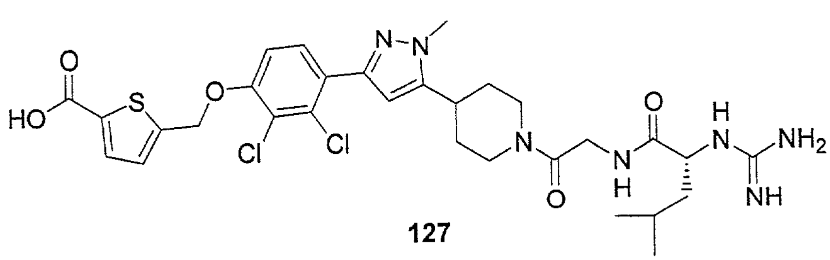
Example 49



126

Title compound **126** was prepared from **98** according to the procedure of Example 48b,c except for using *N*-(4-hydroxymethyl-phenyl)acetamide as a reagent instead of **122.** ES (+) MS m/e = 685 (M+1).

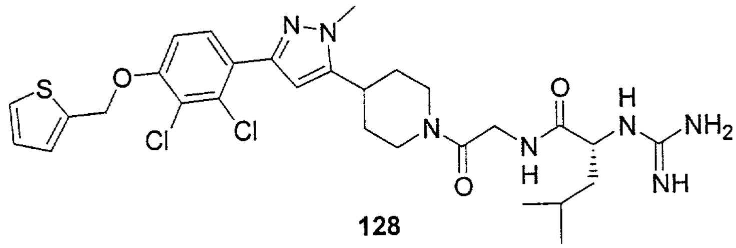
Example 50



127

Title compound **127** was prepared from **98** according to the procedure of Example 48b,c except for using 5-hydroxymethyl-thiophene-2-carboxylic acid as a reagent instead of **122.** ES (+) MS m/e = 678 (M+1).

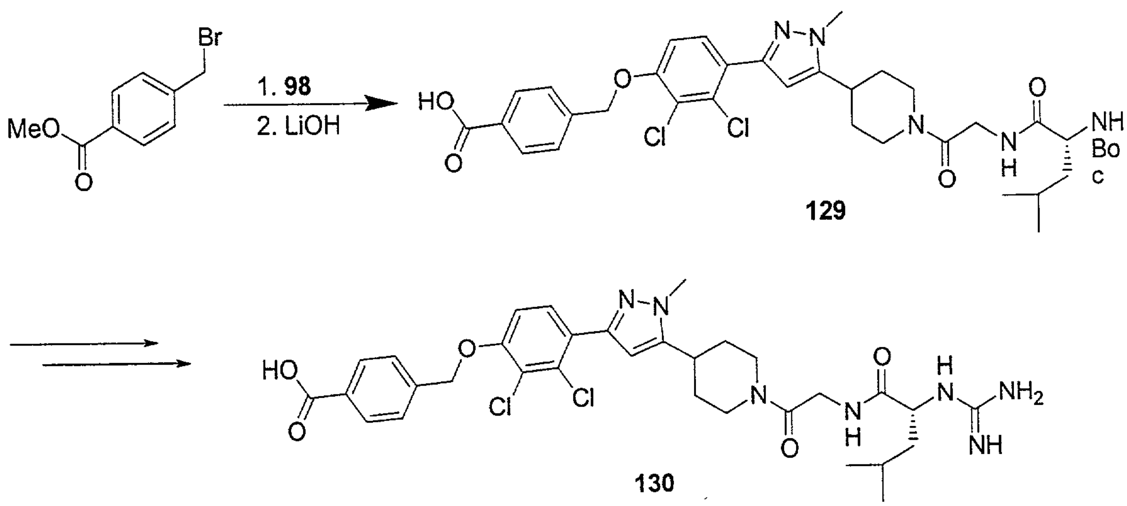
Example 51



128

Title compound **128** was prepared from **98** according to the procedure of Example 48b,c except for using thiophen-2-yl-methanol as a reagent instead of **122.**

Example 52



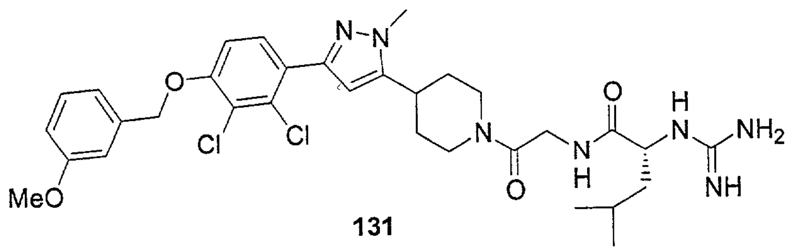
a) A heterogeneous solution containing **98** (75 mg, 0.12 mmol), 4-bromomethyl-benzoic acid methyl ester (43 mg, 0.19 mmol), and potassium carbonate (35 mg, 0.25 mmol) in dry DMF (0.5 mL) was heated to 60°C for 18 h. The reaction mixture was cooled to room temperature and then partitioned between water (10 mL) and ethyl acetate (10 mL). The aqueous layer was washed with ethyl acetate

(2 × 10 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure.

b) The resulting residue was dissolved in THF (0.5 mL) and 1M LiOH (2 mL) and heated to 60°C for 18 h. The reaction mixture was cooled, concentrated under reduced pressure, and then purified by RP HPLC to afford **129** (36 mg, 39%). ES (+) MS m/e = 730 (M+1).

c) Title compound **130** was prepared according to the procedure of Example 16j except for using **129** as a reagent instead of **27.** ES (+) MS m/e = 672 (M+1).

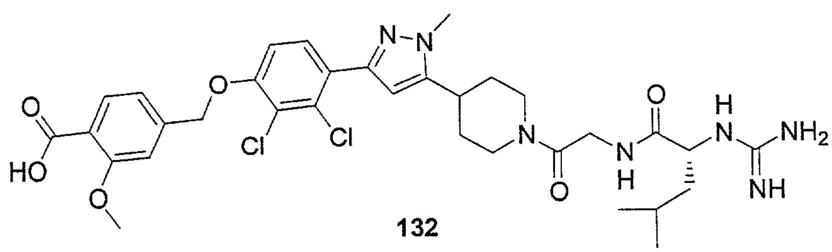
Example 53



131

Title compound **131** was prepared according to the procedure of Example 52a,c except for using 1-bromomethyl-3-methoxybenzene as a reagent instead of 4-(bromomethyl)benzoic acid methyl ester. ES (+) MS m/e = 658 (M+1).

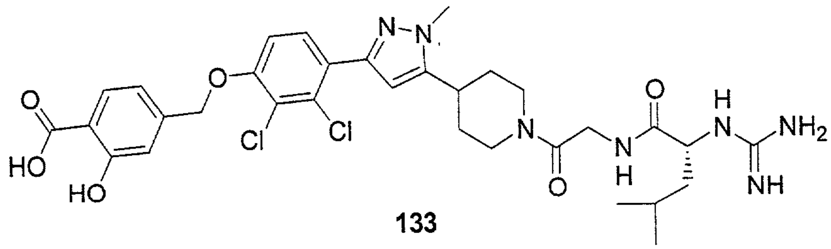
Example 54



132

Title compound **132** was prepared according to the procedure of Example 52a-c except for using 4-bromomethyl-3-methoxybenzoic acid methyl ester as a reagent instead of 4-(bromomethyl)benzoic acid methyl ester. ES (+) MS m/e = 702 (M+1).

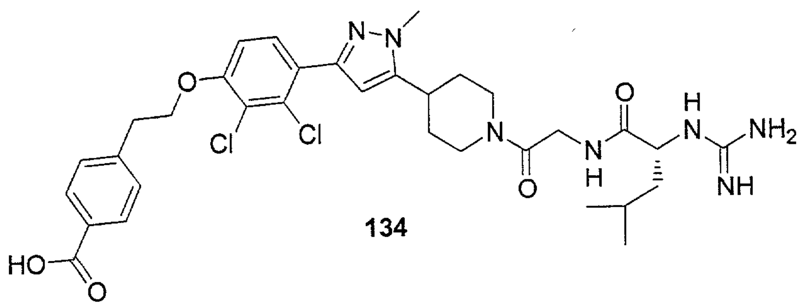
Example 55



133

Title compound **133** was prepared according to the procedure of Example 52a,c except for using 4-bromomethyl-2-(*tert*-butyldimethylsilanyloxy)benzoic acid *tert*-butyldimethylsilyl ester as a reagent instead of 4-(bromomethyl)benzoic acid methyl ester. ES (+) MS m/e = 688 (M+1).

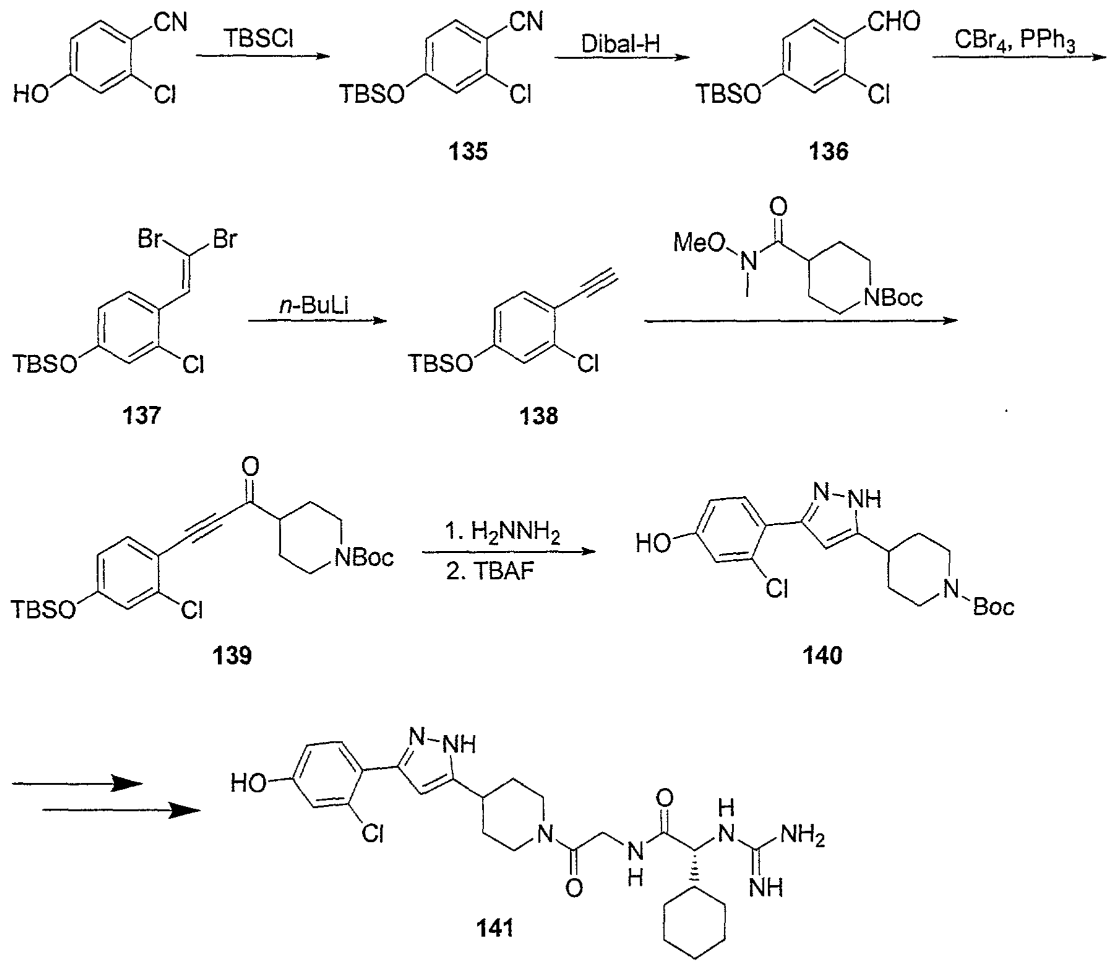
Example 56



134

Title compound **134** was prepared according to the procedure of Example 52a,c except for using 4-(2-bromoethyl)benzoic acid as a reagent instead of 4-bromomethyl-benzoic acid methyl ester. ES (+) MS m/e = 686 (M+1).

Example 57



a) To a solution containing 2-chloro-4-hydroxy-benzonitrile (9.5 g, 61.9 mmol) and imidazole (4.63 g, 68.0 mmol) in dry DMF (120 mL) at room temperature was added *tert*-butyldimethylsilyl chloride (10.3 g, 68.0 mmol). The resulting mixture was stirred for 2 h and then partitioned between water (200 mL) and ethyl acetate (200 mL). The aqueous layer was washed with ethyl acetate (2 × 200 mL); the combined organic layer was dried over $MgSO_4$, filtered through a short plug of silica gel, and concentrated under reduced pressure to afford **135** (16.5 g, 99%). ES (+) MS m/e = 268 (M+1).

b) To a solution containing **135** (16.5 g, 61.6 mmol) in dichloromethane (200 mL) at -78°C was added diisobutylaluminum hydride (1.5M in toluene, 54.0 mL, 81 mmol) dropwise. The reaction mixture was warmed to room temperature and stirred for 30 min. The reaction was quenched by slow addition of isopropanol, diluted with 1M HCl (100 ml) and stirred for 1 h. The aqueous layer was

washed with ethyl acetate (2 × 200 mL); the combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated to afford **136** (15.9 g, 95%). ES (+) MS m/e = 271 (M+1).

c) To a solution containing **136** (10.7 g, 39.3 mmol) and carbon tetrabromide (13.0 g, 39.3 mmol) in dichloromethane (150 mL) was added triphenylphosphine (20.6 g, 78.6 mmol). The resulting mixture was stirred at room temperature and then partitioned between water (300 mL) and ethyl acetate (300 mL). The aqueous layer was washed with ethyl acetate (2 × 150 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was diluted with hexanes and the resulting precipitate was removed by filtration. Concentration of the filtrate under reduced pressure provided **137** (11.3 g, 67%).

d) To a solution containing **137** (11.3 g, 26.4 mmol) in THF (100 mL) at -78°C was added *N*-butyllithium (1.6M in hexanes, 33.0 mL, 52.8 mmol). The reaction mixture was warmed to 0°C, stirred for 30 min, and then partitioned between water (200 mL) and ethyl acetate (200 mL). The aqueous layer was washed with ethyl acetate (2 × 200 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford crude **138** (7.0 g, 99%).

e) To a solution containing **138** (0.75 g, 2.8 mmol) in THF (11 mL) at -78°C was added lithium bis(trimethylsilyl)amide (1.0M in THF, 3.0 mL, 3.0 mmol). Upon complete addition, a solution of 4-(methoxymethylcarbamoyl)piperidine-1-carboxylic acid *tert*-butyl ester (0.76 g, 2.8 mmol) in THF (3 mL) was added and the resulting mixture was allowed to warm to 0°C. The reaction mixture was stirred for 30 minutes, warmed to room temperature, and then partitioned between saturated ammonium chloride (50 mL) and ethyl acetate (50 mL). The aqueous layer was washed with ethyl acetate (2 × 50 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford **139** (1.3 g, 97%).

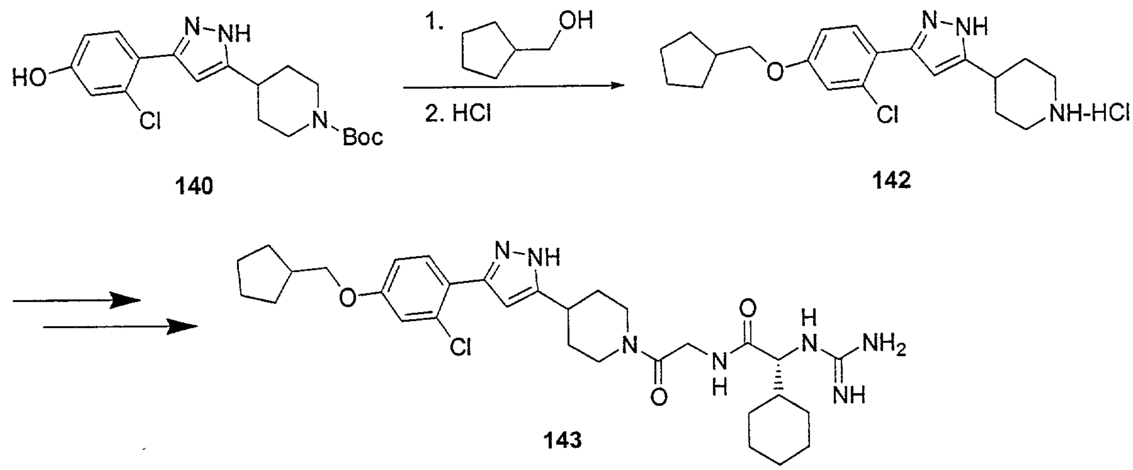
f) To a solution containing **139** (6.0 g, 12.5 mmol) in ethanol (12 mL) was added hydrazine (1 mL). The resulting mixture was stirred at room temperature for 1 h and then partitioned between water (50 mL) and ethyl acetate (50 mL). The aqueous layer was washed with ethyl acetate (3 × 50 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure.

To the resulting residue in THF (12 mL) at 0°C was added tetrabutylammonium fluoride (1.0M in THF, 13 mL, 13 mmol) dropwise. After 30 min the solution was partitioned between water (50 mL) and ethyl acetate (50 mL). The aqueous layer was washed with ethyl acetate (3 × 50 mL); the combined organic

layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2: 50 to 75% ethyl acetate in hexanes) to afford **140** (3.43 g, 72%).

g) Title compound **141** was prepared according to the procedure of Example 16g,i,j except for using **140** as a reagent instead of **24**. ES (+) MS m/e = 516 (M+1).

Example 58

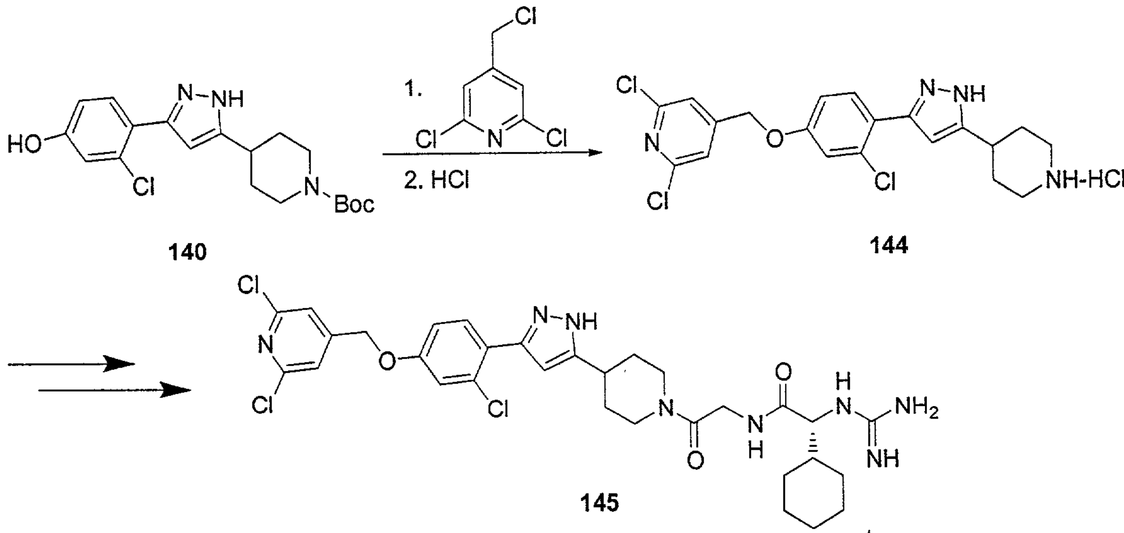


140 **142** **143**

a) To a solution containing **140** (80 mg, 0.21 mmol), triphenylphosphine (56 mg, 0.21 mmol), and cyclopentanemethanol (23 μL, 0.21 mmol) in dichloromethane (1 mL) was added di-*tert*-butyl-azodicarboxylate (49 mg, 0.21 mmol). The resulting mixture was stirred for 18 h, concentrated under reduced pressure, and then purified by flash chromatography (SiO_2: 50% ethyl acetate in hexanes). To the resulting residue in dioxane (0.5 mL) was added HCl/dioxane (4.0N, 1 mL). After stirring for 1 h, the mixture was concentrated under reduced pressure to afford **142** (64 mg, 76%). ES (+) MS m/e = 360 (M+1).

b) Title compound **143** was prepared according to the procedure of Example 16i,j except for using **142** as a reagent instead of **25**. ES (+) MS m/e = 598 (M+1).

Example 59



140

144

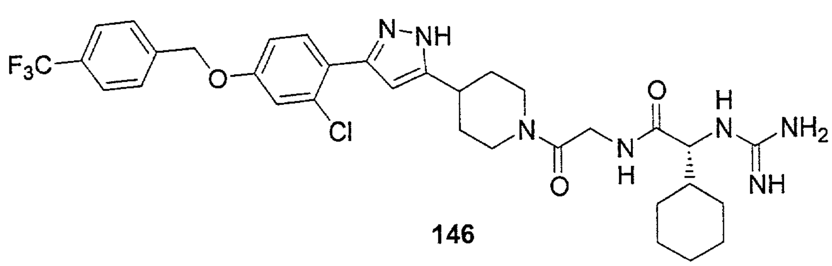
145

a) A solution of **140** (100 mg, 0.26 mmol), 2,6-dichloro-4-chloromethyl-pyridine hydrochloride (75 mg, 0.32 mmol), and cesium carbonate (258 mg, 0.79 mmol) in DMSO (1 mL) was stirred at 65°C for 1 hour. The reaction mixture was cooled to room temperature and then partitioned between water (10 mL) and ethyl acetate (10 mL). The aqueous layer was washed with ethyl acetate (3 × 10 mL); the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2: 50 to 75% ethyl acetate in hexanes).

To a solution of the residue in dioxane (0.5 mL) was added HCl/dioxane (4.0N, 1 mL). The reaction mixture was stirred for 1 hour and then concentrated under reduced pressure to afford **144** (36 mg, 27%). ES (+) MS m/e = 439 (M+3).

b) Title compound **145** was prepared according to the procedure of Example 16i,j except for using **144** as a reagent instead of **25**. ES (+) MS m/e = 675 (M+1).

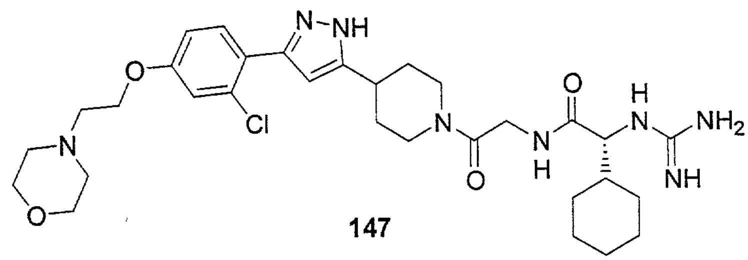
Example 60



146

Title compound **146** was prepared according to the procedure of Example 59a,b except for using 1-bromomethyl-4-trifluoromethylbenzene as a reagent instead of 2,6-dichloro-4-chloromethylpyridine hydrochloride. ES (+) MS m/e = 674 (M+1).

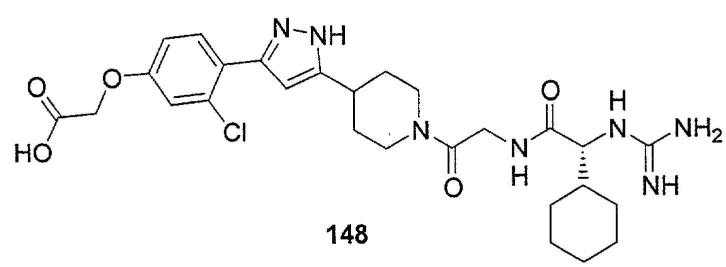
Example 61



147

Title compound **147** was prepared according to the procedure of Example 59a,b except for using 4-(2-chloro-ethyl)-morpholine hydrochloride as a reagent instead of 2,6-dichloro-4-chloromethyl-pyridine hydrochloride. ES (+) MS m/e = 629 (M+1).

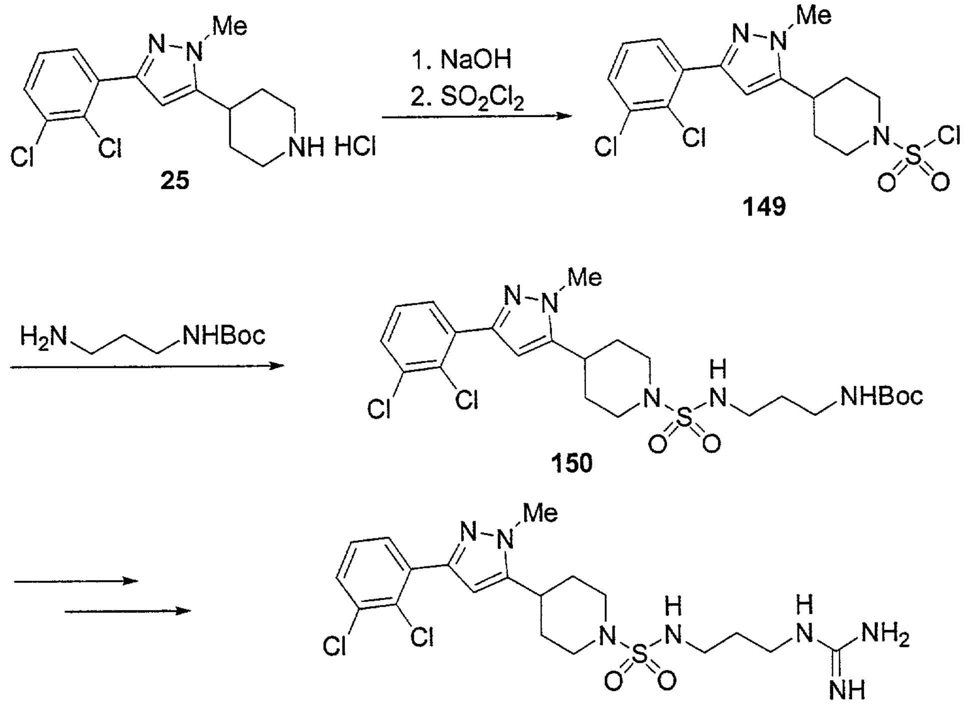
Example 62



148

Title compound **148** was prepared according to the procedure of Example 52a-c except for using methyl bromoacetate as a reagent instead of 4-bromomethyl-benzoic acid methyl ester. ES (+) MS m/e = 574 (M+1).

Example 63



25, 149, 150, 151

a) To a solution of **25** (1.0 g, 2.9 mmol) in dichloromethane (10 mL) was added 1M NaOH (10 mL). The aqueous layer was extracted with dichloromethane (2 x 10 mL); the combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and azeotroped with benzene (3 × 5 mL) to give the free amine (891 mg, 99%).

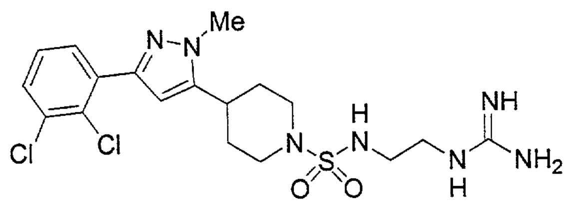
To a solution of sulfuryl chloride (1.0M in dichloromethane, 2.7 mL, 2.7 mmol) in dichloromethane (20 mL) at –78°C under nitrogen was added the free amine in dichloromethane (5 mL) and triethylamine (443 μL, 3.2 mmol) dropwise. The reaction was stirred at –78°C for 2h, the dry ice bath was removed, and stirring continued at room temperature overnight. The mixture was diluted with dichloromethane (10 mL), partitioned with 1M HCl (20 mL) and separated. The aqueous layer was extracted with dichloromethane (3 × 10 mL); and the combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield **149** (809 mg, 72%). ES (+) MS m/e = 410 (M+1).

b) To **149** (60 mg, 0.15 mmol) in dichloromethane (1 mL) was added triethylamine (23 μL, 0.17 mmol) followed by *N*-Boc-propanediamine (39 mg, 0.23 mmol). The reaction was stirred overnight at room temperature and then was partitioned between dichloromethane (5 mL) and 1M HCl (5 mL). The aqueous layer was extracted with dichloromethane (2 × 5 mL); the combined organic layer was

dried over Na_2SO_4 and concentrated under reduced pressure to provide **150**. ES (+) MS m/e = 490 (M-55).

c) Title compound **151** was prepared according to the procedure of Example 16j except for using **150** as a reagent instead of **27**. ES (+) MS m/e = 488 (M+1).

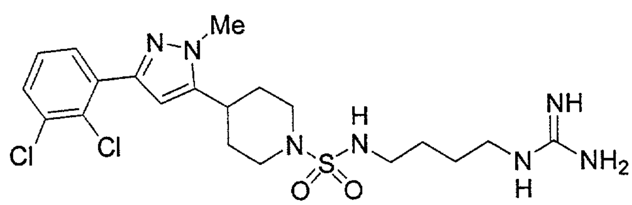
Example 64



152

Title compound **152** was prepared from **149** according to the procedure of Example 63b,c except for using *N*-Boc-ethylenediamine as a reagent instead of *N*-Boc-propanediamine. ES (+) MS m/e = 474 (M+1).

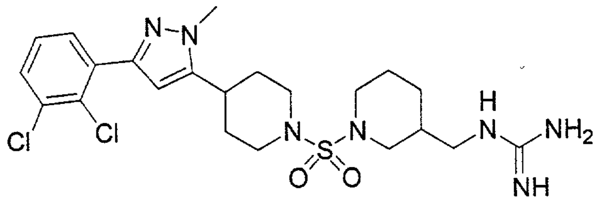
Example 65



153

Title compound **153** was prepared from **149** according to the procedure of Example 63b,c except for using *N*-Boc-butanediamine as a reagent instead of *N*-Boc-propanediamine. ES (+) MS m/e = 502 (M+1).

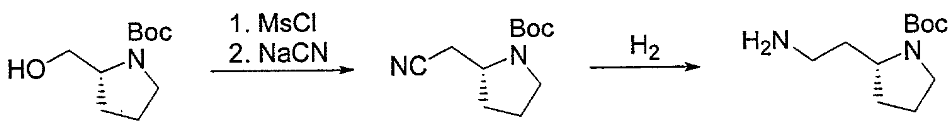
Example 66



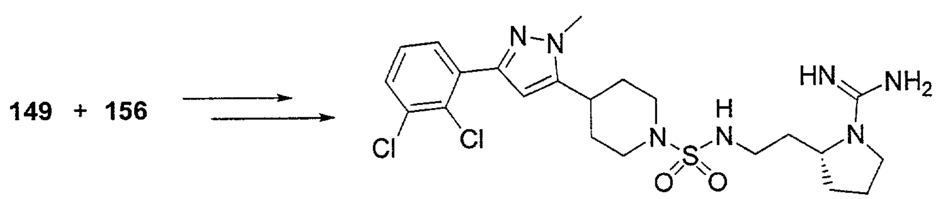
154

Title compound **154** was prepared from **149** according to the procedure of Example 63b,c except for using 3-aminomethyl-*N*-Boc-piperidine as a reagent instead of *N*-Boc-propanediamine.

Example 67



155 **156**



157

a) To a solution of Boc-D-prolinol (3.05 g, 15.1 mmol) in dichloromethane (30 mL) at 0°C was added triethylamine (2.66 mL, 19 mmol) followed by mesyl chloride (1.35 mL, 17.5 mmol). The reaction mixture was warmed to room temperature, stirred for 2 h, and then partitioned between dichloromethane and water. The aqueous layer was extracted with dichloromethane; the organic layer was extracted with 1M HCl, dried over $MgSO_4$, and concentrated *in vacuo*.

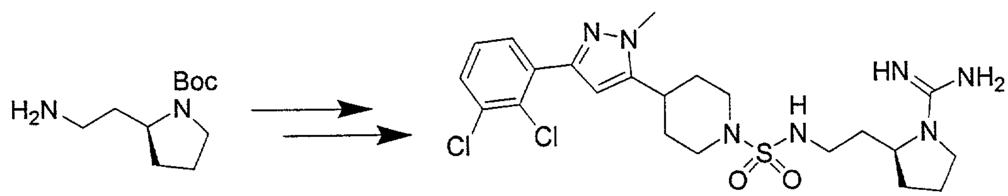
To the resulting yellow oil in DMF (20 mL) at 55°C was added NaCN (1.47 g, 30 mmol). The reaction was stirred overnight and monitored by TLC. After 15 h, the reaction was only 50% complete. To the solution was added KCN (1.3 g, 20 mmol) and the reaction was stirred an additional 24 h. The reaction

mixture was cooled to room temperature and then partitioned between dichloromethane and water. The organic layer was extracted with water, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by flash chromatography (SiO_2: 20% ethyl acetate in hexanes) provided **155** (1.75 g, 55%) as a colorless oil.

b) To a solution of **155** (1.75 g, 8.3 mmol) in saturated ammonia/ethanol (30 mL) was added Raney nickel (slurry in water, 3 mL). The solution was shaken on a Parr hydrogenator (H_2, 40 psi) for 3 h, filtered through Celite, and then washed with methanol. The filtrate was dried over Na_2SO_4 and concentrated *in vacuo* to provide (1.75 g, 100%) of **156**.

c) Title compound **157** was prepared from **149** according to the procedure of Example 63b,c except for using (*R*)-2-aminoethyl-*N*-Boc-pyrrolidine as a reagent instead of *N*-Boc-propanediamine. ES (+) MS m/e = 514 (M+1).

Example 68



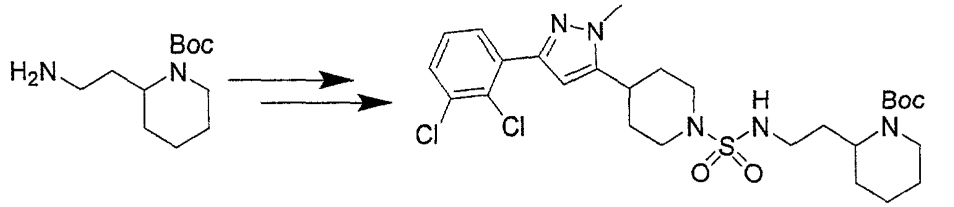
158

159

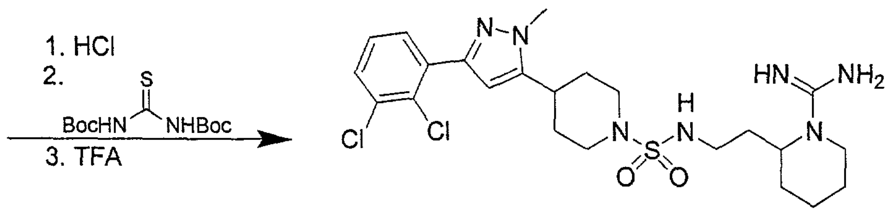
a) Compound 158 was prepared according to the procedure of Example 67a,b except for using Boc-L-prolinol as a reagent instead of Boc-D-prolinol.

b) Title compound 159 was prepared from 149 according to the procedure of Example 63b,c except for using (*S*)-2-aminoethyl-*N*-Boc-pyrrolidine as a reagent instead of *N*-Boc-propanediamine. ES (+) MS m/e = 600 (M+1).

Example 69



160



161

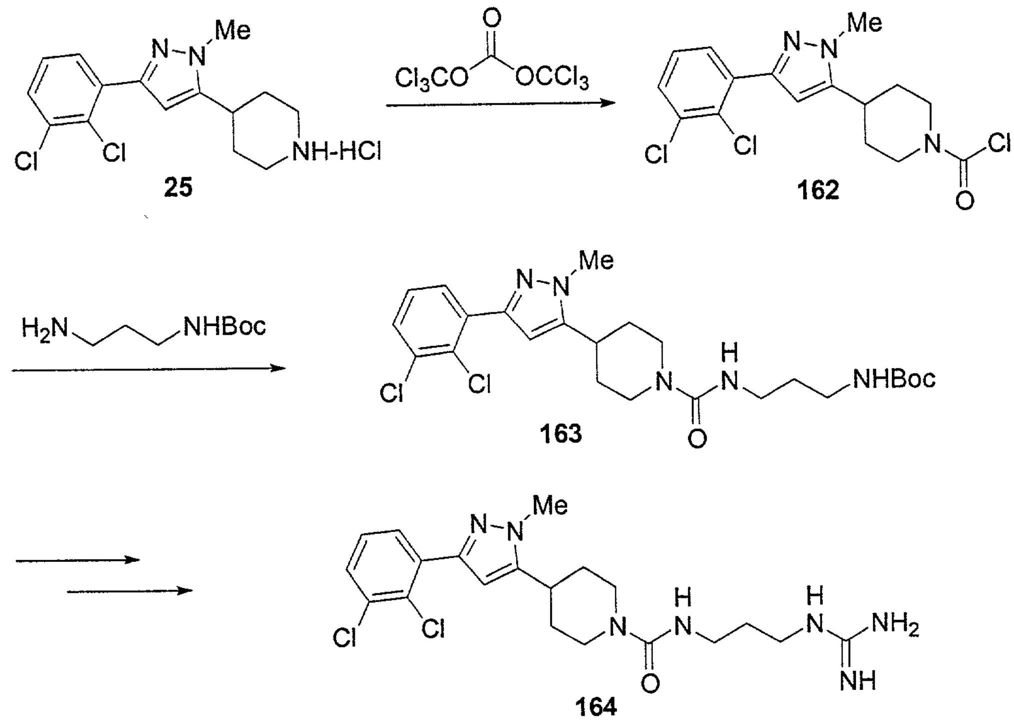
a) Compound **160** was prepared from **149** according to the procedure of Example 63b except for using 2-aminoethyl-*N*-Boc-piperidine as a reagent instead of *N*-Boc-propanediamine. ES (+) MS *m/e* = 600 (M+1).

b) A solution of **160** in HCl/dioxane (4N, 1 mL) was stirred at room temperature for 30 min. The solvent was removed under reduced pressure to provide the desired amine as the hydrochloride salt which was used without purification.

To the hydrochloride salt (107 mg, 0.2 mmol) in dichloromethane (0.5 mL) was added a solution of 2-chloro-*N*-methylpyridinium iodide (77 mg, 0.3 mmol), *N,N'-bis*-Boc-thiourea (83 mg, 0.3 mmol), and triethylamine (42 μL, 0.3 mmol) in dichloromethane (0.5 mL). The reaction was stirred at room temperature overnight and the solvent was removed under reduced pressure.

The protected guanidine was dissolved in TFA/dichloromethane (1:1, 1 mL) and stirred at room temperature for 3h. The solvent was removed under reduced pressure to afford the crude guanidine as the trifluoroacetate salt. The crude material was purified by RP HPLC to provide **161**. ES (+) MS m/e = 542 (M+1).

Example 70

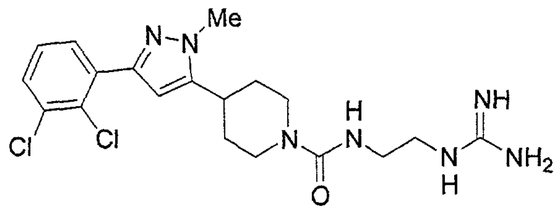


a) To a solution of **25** (4.5 g, 12.9 mmol) and triethylamine (3.8 mL, 27.1 mmol) in dichloromethane (60 mL) was added portionwise triphosgene (3.8 g, 12.9 mmol). The reaction was stirred at room temperature for 30 minutes and then partitioned between dichloromethane (20 mL) and 1M HCl (50 mL). The aqueous layer was extracted with dichloromethane (3 X 50 mL); the combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to provide **162** (4.8 g, 100%). ES (+) MS m/e = 372 (M+1).

b) To a solution of **162** (74 mg, 0.20 mmol) in dichloromethane (1 mL) was added triethylamine (56 μL, 0.40 mmol) followed by *N*-Boc-propanediamine (35 mg, 0.20 mmol). The reaction was stirred overnight at room temperature and then partitioned between dichloromethane (5 mL) and 1M HCl (5 mL). The aqueous layer was extracted with dichloromethane (2 × 5 mL); the combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to provide **163**. ES (+) MS m/e = 510 (M+1).

c) Title compound **164** was prepared according to the procedure of Example 16j except for using **163** as a reagent instead of **27**. ES (+) MS m/e = 451 (M+1).

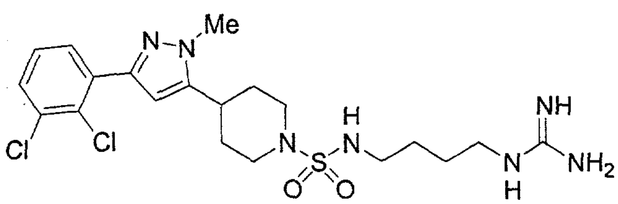
Example 71



165

Title compound **165** was prepared from **162** according to the procedure of Example 70b,c except for using *N*-Boc-ethylenediamine as a reagent instead of *N*-Boc-propanediamine. ES (+) MS m/e = 437 (M+1).

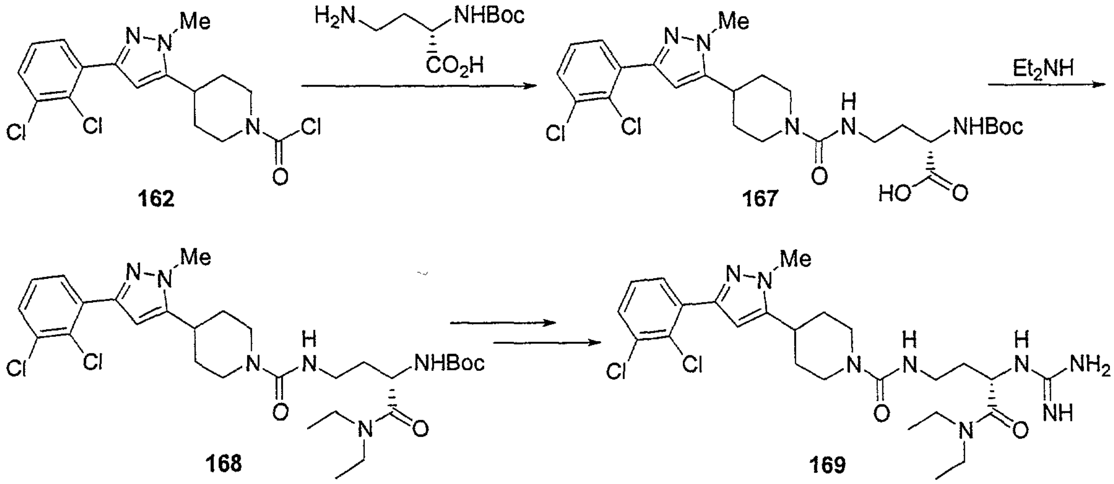
Example 72



166

Title compound **166** was prepared from **162** according to the procedure of Example 70b,c except for using *N*-Boc-butanediamine as a reagent instead of *N*-Boc-propanediamine. ES (+) MS m/e = 465 (M+1).

Example 73

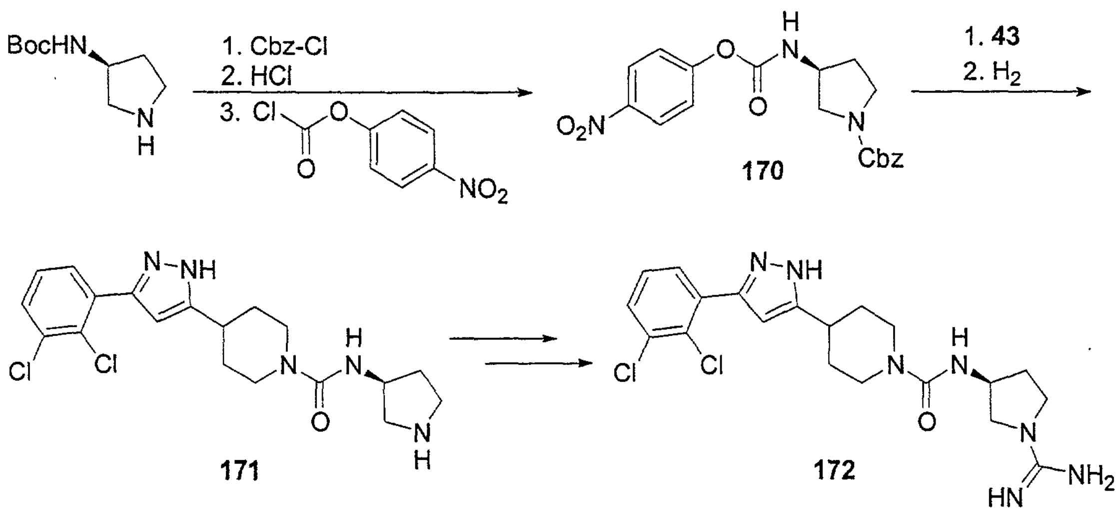


a) To a solution of **162** (4.8 g, 12.9 mmol) in THF (40 mL) was added a solution of *N*-Boc-diaminobutyric acid (3.4 g, 15.5 mmol) in water/triethylamine (1:1, 20 mL). The reaction was stirred at room temperature for 2h and then the mixture was partitioned between ethyl acetate (100 mL) and 1M HCl (100 mL). The aqueous layer was extracted with ethyl acetate (3 × 70 mL); the combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford 7.0 g (99%) of **167**. ES (+) MS m/e = 554 (M+1).

b) To a solution of **167** (63 mg, 0.11 mmol) and triethylamine (38 μL, 0.27 mmol) in dichloromethane (0.5 mL) was added 1,1′-carbonyldiimidazole (23 mg, 0.14 mmol). After 1 h, diethylamine (14 μL, 0.14 mmol) was added and the reaction was stirred overnight. The solvent was removed under reduced pressure to yield **168** which was used without further purification. ES (+) MS m/e = 609 (M+1).

c) Title compound **169** was prepared according to the procedure of Example 16j except for using **168** as a reagent instead of **27**. ES (+) MS m/e = 551 (M+1).

Example 74



a) To a solution of (3*S*)-*N*-Boc-aminopyrrolidine (2.0 g, 10.7 mmol) and triethylamine (1.6 mL, 11.8 mmol) in dichloromethane (45 mL) was added benzyl chloroformate (1.5 mL, 10.2 mmol). The reaction was stirred at room temperature for 2h, then partitioned between water (20 mL) and dichloromethane. The aqueous layer was extracted with dichloromethane (2 × 20 mL); the combined organic layer was washed with 1M HCl (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure to yield the desired *bis*-carbamate (2.9 g, 88%).

A solution of the *bis*-carbamate (500 mg, 1.6 mmol) in HCl/dioxane (4N in dioxane, 6 mL) was stirred at room temperature for 30 minutes. The solvent was removed under reduced pressure to provide the amine (400 mg, 100%) as the hydrochloride salt.

To the amine (400 mg, 1.6 mmol) and pyridine (0.29 mL, 3.6 mmol) in dichloromethane (6 mL) at 0°C was added *p*-nitrophenyl chloroformate (299 mg, 1.5 mmol). The reaction mixture was stirred at 0°C for 2 h and then stirred at room temperature overnight. The solution was diluted and partitioned between dichloromethane (10 mL) and 1M HCl (15 mL). The aqueous layer was extracted with dichloromethane (3 × 15 mL); the combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude material by flash chromatography (SiO_2: 40% ethyl acetate in hexanes) provided **170** (190 mg, 33%). ES (+) MS m/e = 386 (M+1).

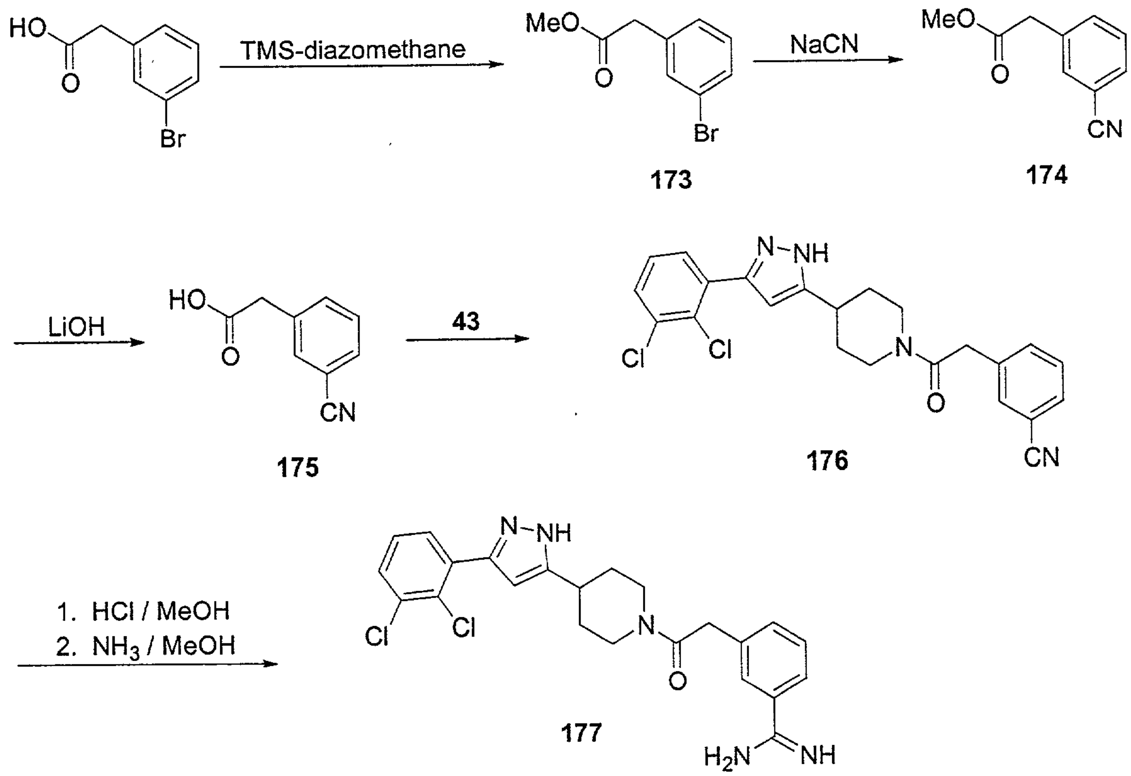
b) A solution of **170** (177 mg, 0.46 mmol) and 43 in pyridine (2.5 mL) was stirred at reflux for 3 h. The reaction mixture was cooled to room temperature and then partitioned between ethyl acetate

(10 mL) and 1M HCl (10 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL); the combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield the urea (337 mg) which was used without purification.

To a solution of urea in methanol/ethyl acetate (1:1, 5 mL) was added 10% palladium on carbon (75 mg). The suspension was stirred under an atmosphere of hydrogen at room temperature for 2 h. The reaction mixture was filtered through Celite and washed with methanol (2 × 10 mL). The filtrate was concentrated under reduced pressure to provide **171** (190 mg) as an oil. ES (+) MS m/e = 408 (M+1).

c) Title compound **172** was prepared according to the procedure of Example 16j except for using **171** as a reagent instead of **27**. ES (+) MS m/e = 450 (M+1).

Example 75



a) To 3-bromophenyl acetic acid (5.1 g, 23.7 mmol) in toluene (60 mL) and methanol (6 mL) was added dropwise TMS•diazomethane (2.0M in hexanes, 15 mL, 30 mmol). The solvent was removed under reduced pressure to provide **173** (5.45 g, 100%) as a yellow oil.

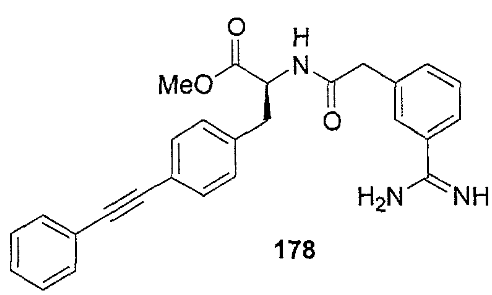
b) To a solution of **173** (3.43 g, 15 mmol) in propionitrile (22 mL) was added NaCN (1.47 g, 30 mmol), CuI (0.29 g, 1.5 mmol), and $Pd(PPh_3)_4$ (0.86 g, 0.75 mmol). The reaction mixture was heated to reflux and stirred overnight. The solution was cooled, filtered through Celite, and then partitioned between ethyl acetate and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to provide **174** (0.52 g, 20%). ES (+) MS m/e = 176 (M+1).

c) To a solution of **174** (0.11 g, 0.61 mmol) in THF (1 mL) and water (0.25 mL) was added lithium hydroxide monohydrate (31 mg, 0.75 mmol). After 3h the reaction mixture was partitioned between ethyl acetate and 1N HCl. The aqueous layer was extracted twice with ethyl acetate, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to provide **175** (82 mg, 84%) as a white solid.

d) To a solution of **43** (200 mg, 0.6 mmol) in dichloromethane was added **175** (82 mg, 0.51 mmol), EDC (115 mg, 0.6 mmol), HOBt (92 mg, 0.6 mmol), and triethylamine (0.15 mL, 1.1 mmol). After stirring the reaction mixture overnight the solution was partitioned between dichloromethane and water. The aqueous layer was extracted with dichloromethane; the organic layer was extracted with 1N HCl, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography (SiO_2: 100% ethyl acetate) provided **176** (166 mg, 77%). ES (+) MS m/e = 440 (M+1).

e) To **176** (70 mg, 0.16 mmol) was added saturated HCl in MeOH (3 mL). The reaction was warmed to 60 °C, stirred for 1.5h, and then concentrated *in vacuo*. The crude residue was dissolved in ammonia/MeOH (2M, 3 mL), warmed to 60°C, and stirred for 1.5 h. The solvent was removed *in vacuo* and the material was purified by RP-HPLC to provide **177** as a white solid. ES (+) MS m/e = 457 (M+1).

Example 76



178

Title compound **178** was prepared according to the procedure of Example 75d,e except for using **1** as a reagent instead of **43**. ES (+) MS m/e = 440 (M+1).

Example 77. Scintillation Proximity Assay

Compounds are shown to inhibit binding of IL-2 to IL-2Rα by the following method:

1. IL-2 receptor alpha is expressed in SF9 insect cells and purified by his-tag affinity chromatography. The protein is then labeled with the NHS-ester of biotin by combining protein with four molar equivalents of biotin-LC-NHS (Pierce) in 50 mM Na_2CO_3 buffer at pH 9. The mixture is incubated for one hour at room temperature and purified by size-exclusion chromatography (Nap5, Pierce) into phosphate-buffered saline (PBS).

2. IL-2 is expressed in *E. coli* cells and purified by standard procedures. Protein is then labeled with (^{3}H)-propionic acid NHS ester (Amersham) by combining 10 nmol IL-2 with 1 mCi propionic acid in 50 mM Na_2CO_3 buffer at pH 9. The mixture is incubated for one hour at room temperature and purified by size-exclusion chromatography (Nap 5, Pierce) into SuperBlock/PBS (Pierce).

3. Biotinylated-IL-2 receptor alpha is bound to streptavidin-coated scintillation beads (Amersham) by adding 100 nM protein to 10 mL of beads per 96-well plate. The mixture is incubated for 20 minutes at room temperature, centrifuged, and decanted. The beads are then resuspended in 1 mL of SuperBlock/PBS. 10 μL are aliquoted to each well in a clear-bottom 96-well plate (Wallac).

4. The compounds to be tested are suspended in DMSO to a final concentration of 100 mM. The compounds are then serially diluted by three-fold dilutions in DMSO and 1.2 μL of each dilution is then transferred to a clean, 96-well plate. The DMSO solutions are mixed with 120 μL of a solution containing 8 nM 3H-labeled IL-2. 90 μL of this mixture is then transferred to the 96-well plate containing IL-2 receptor alpha-coated beads from step (3). The mixture is incubated for 20 min.

5. Luminescence is read in a Wallac Microbeta Scintillation Counter. Luminescence arises from binding of 3H-labeled IL-2 to the scintillant-containing beads; reduction in the luminescence is due to inhibition of the protein-protein interaction by binding of the compounds.

Compounds of this invention are active in this assay.

Example 78. Inhibition of STAT5 phosphorylation

Cell Culture

1. CTLL-2 cells are grown to approximately $1x10^6$ cells/mL in a 37°C, 5% CO_2, incubator; using complete media [RPMI 1640 (liquid) with L-glutamine, 10mM HEPES, 1mM sodium pyruvate, 4.5 g/L

glucose, 1.5 g/L sodium bicarbonate, 10% fetal bovine serum, 2-mercaptoethanol, and antibiotic/antimycotic (10,000 units/mL penicillin G sodium, 10,000 μg/mL streptomycin sulfate, 25 μg/mL amphotericin B)] plus 5 ng/mL IL-2.

2. When cells are close to $1x10^6$ cells/mL, they are starved without IL-2 overnight. The cells are spun down (1100 rpm for 5 min), resuspended in IL-2-free complete media to wash, spun down again, resuspended in IL-2-free complete media to the same initial volume, and incubated overnight.

Sample Testing

1. The compounds are diluted in DMSO into Eppendorf tubes.
2. 2 μL of each compound dilution is aliquoted into a 15 ml conical tube. 1478 μL complete media (no IL-2) is added. 20 μL diluted IL-2 (0.01 ng/μL) is added (the final concentration is 0.1 ng/ml).
3. The starved cells are counted, spun down, and resuspended (in pre-warmed complete media) to $8x10^6$ cells/mL. 500 μL is aliquoted to each tube, to give $4x10^6$ cells per tube, 2 mL final volume.
4. The tubes are incubated for 30 min in a 37°C incubator.

Cell Extract Preparation

1. The cells are spun down (1100 rpm for 5 min), and excess media removed. The cell pellet is resuspended and lysed with 30 μL of M-PER buffer (from Pierce) containing protease inhibitors [using either (a) 10 μg/mL aprotinin, 10 μg/mL antipapain, 5 μg/mL leupeptin, and 1 mg/mL Pefablock SC, or (b) complete mini protease inhibitor cocktail tablets from Roche (catalog # 183615] and phosphatase inhibitors [50 mM NaF, 80 mM sodium glycerophosphate, 2 mM sodium vanadate (heat activated stock solution)], and is then placed on ice.
2. The suspensions are transferred to Eppendorf tubes, submitted to a freeze/thaw cycle (dry ice/-room temperature), and spun in a microcentrifuge at full speed for 15 min in a cold room.
3. The supernatant is transferred to a new tube; and protein concentration quantified using Bio-Rad protein assay dye reagent (Bio-Rad Laboratories, catalog #500-0006)

Gel Electrophoresis and Western Blotting

1. 20 μg of protein extract is mixed with sample buffer and reducing agent (Invitrogen/Novex), then heated for 10 min at 70°C. Samples are spun in a microcentrifuge briefly to collect all sample at the bottom of the tube, then loaded onto a mini-protein gel (Invitrogen/Novex) (using either 4-12% Bis-Tris

or 10% Bis-Tris gels). Full Range Rainbow Markers (Amersham) are loaded in one lane. 500 µL antioxidant (Invitrogen/Novex) is added into then inner buffer chamber, and the gel electrophoresed at 180 V for 1 h in 1× MOPS running buffer (Invitrogen/Novex).

2. The gel is transferred to a PVDF membrane (Invitrogen/Novex) according to the manufacturer's instructions. This entails pre-wetting the PVDF membrane in methanol, then equilibrating in transfer buffer with either 10% (one gel) or 15% (two gels) methanol added, and transferring at 35V for 1 h. The gel is cut horizontally at around the 50K marker to obtain a top and a bottom half.

3. The membrane is blocked with 5% non-fat dry milk (NFDM) in 1× TBS + 0.05% Tween 20 (TBST) for 1 h at room temperature on an orbital shaker.

4. The primary antibodies are diluted in 5% NFDM in TBST. For the top half, Phospho-STAT5 (Upstate Biotech) at 1:1000 is used. For the bottom half, PCNA (Pharmingen) at 1:2000, or cdc2 (Transduction Labs) at 1:1000 is used.

5. The membranes are incubated overnight at 4°C on a nutator; and the blot washed 3-4 times (for about 15 min each) with TBST.

Secondary antibodies and development procedures

1. HRP-conjugated rabbit anti-mouse antibody (Zymed) is diluted 1:1000 in 5% NFDM in TBST, and incubated for several hours at room temperature on a nutator. The blot is washed 3-4 times (for about 15 min each) with TBST. The blot is developed using ECL Plus Western blotting detection reagents (Amersham): The reagents are mixed in a 1:40 dilution, the membrane dried briefly, and about 5 mL of reagent solution applied to the protein side of the membrane; which is then incubated on a flat surface with no agitation for 5 min, excess liquid dripped off, and the membrane wrapped in Saran wrap. The membrane is exposed to Kodak BioMax MR film for 10 sec to 5 min; and the film developed.

2. AP-conjugated donkey anti-mouse antibody (Jackson Immunoresearch) is diluted 1:2000 or 1:400 in 5% NFDM in TBST, then incubated for several hours at room temperature on a nutator. The blot is washed 3-4 times (for about 15 min each) with TBST. The blot is developed using ECF substrate for Western blotting (Amersham): The reagents are mixed, and about 2 mL applied to the protein side of the wet membrane; which is then incubated for 2-20 min on a flat surface with no agitation, dried thoroughly, and scanned scan on a phosphorimager (using the fluorescence/fluorescein setting: excitation 532, emission 526 SP.

Compounds of this invention are active in this assay.

While this invention has been described in conjunction with specific embodiments and examples, it will be apparent to a person of ordinary skill in the art, having regard to this disclosure, that equivalents of the specifically disclosed materials and techniques will also be applicable to this invention; and such equivalents are intended to be included within the following claims.